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MODERN PRACTICE OF GAS CHROMATOGRAPHY

MODERN PRACTICE OF GAS CHROMATOGRAPHY

FOURTH EDITION

Edited by

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To

Our
Wives and Families

What is written without effort is in general read without pleasure

—Samuel Johnson (1709–1784)
Johnsonian Miscellanies
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PREFACE

The fourth edition of *Modern Practice of Gas Chromatography* represents a number of changes from the first three editions. First, a number of new contributing authors have been involved. These authors were chosen because of their expertise and active participation in the various areas related to gas chromatography (GC). Second, the contents of the various chapters have been changed so as to be all-inclusive. For example, a discussion of the necessary instrumentation has been included in chapters covering such topics as columns, detectors, fast gas chromatography, and sample preparation. Third, separate chapters are dedicated to gas chromatography/mass spectrometry, sample preparation, fast gas chromatography, optimization and computer assistance, and QA/QC validation of gas chromatographic methods. Another change has been the elimination of several chapters because of their adequate coverage in other texts. The editors are satisfied that this new edition represents an all-inclusive text that may be used for university courses as well as short courses.

No book will please everyone. Each person has certain ideas concerning what should be covered and how much detail should be given to each topic. Coverage of the theory and basics of GC is what we consider necessary to the beginner for this technique and the nomenclature is that most recently recommended by the IUPAC Commission. The techniques and instrumentation section is greatly detailed, and the application chapters cover topics that would be of interest to most people utilizing the gas chromatographic technique.

The editors thank the contributing authors for their cooperation and professionalism, thus making this fourth edition a reality. A special thanks to Dr. Nicholas H. Snow, of Seton Hall University for his contributions over and above the professional level. Most importantly, the editors thank their wives Marjorie and Dee for their interest, encouragement, and cooperation during these many months of preparation. Dr. Grob especially wishes to thank his son, G. Duane Grob for all his assistance and encouragement in the computer aspects of putting this book together.

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Malvern, Pennsylvania
2004

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Nashua, New Hampshire
2004

CHAPTER ONE

Introduction

ROBERT L. GROB

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1.1 HISTORY AND DEVELOPMENT OF CHROMATOGRAPHY
1.2 DEFINITIONS AND NOMENCLATURE
1.3 SUGGESTED READING ON GAS CHROMATOGRAPHY
l.4 COMMERCIAL INSTRUMENTATION
REFERENCES

1.1 HISTORY AND DEVELOPMENT OF CHROMATOGRAPHY

Many publications have discussed or detailed the history and development of chromatography (1–3). Rather than duplicate these writings, we present in Table 1.1 a chronological listing of events that we feel are the most relevant in the development of the present state of the field. Since the various types of chromatography (liquid, gas, paper, thin-layer, ion exchange, supercritical fluid, and electrophoresis) have many features in common, they must all be considered in development of the field. Although the topic of this text, gas chromatography (GC), probably has been the most widely investigated since the early 1970s, results of these studies have had a significant impact on the other types of chromatography, especially modern (high-performance) liquid chromatography (HPLC).

There will, of course, be those who believe that the list of names and events presented in Table 1.1 is incomplete. We simply wish to show a development of an ever-expanding field and to point out some of the important events that were responsible for the expansion. To attempt an account of contemporary leaders of the field could only result in disagreement with some workers, astonishment by others, and a very long listing that would be cumbersome to correlate.

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TABLE 1.1 Development of the Field of Chromatography

Year (Reference)	Scientist(s)	Comments
1834 (4) 1834 (5)	Runge, F. F.	Used unglazed paper and/or pieces of cloth for spot testing dye mixtures and plant extracts
1850 (6)	Runge, F. F.	Separated salt solutions on paper
1868 (7)	Goppelsroeder, F.	Introduced paper strip (capillary analysis) analysis of dyes, hydrocarbons, milk, beer, colloids, drinking and mineral waters, plant and animal pigments
1878 (8)	Schönbein, C.	Developed paper strip analysis of liquid solutions
1897–1903 (9–11)	Day, D. T.	Developed ascending flow of crude petroleum samples through column packed with finely pulverized fuller's earth
1906–1907 (12–14)	Twsett, M.	Separated chloroplast pigment on $CaCO_3$ solid phase and petroleum ether liquid phase
1931 (15)	Kuhn, R. et al.	Introduced liquid–solid chromatography for separating egg yolk xanthophylls
1940 (16)	Tiselius, A.	Earned Nobel Prize in 1948; developed adsorption analyses and electrophoresis
1940 (17)	Wilson, J. N.	Wrote first theoretical paper on chromatography; assumed complete equilibration and linear sorption isotherms; qualitatively defined diffusion, rate of adsorption, and isotherm nonlinearity
1941 (18)	Tiselius, A.	Developed liquid chromatography and pointed out frontal analysis, elution analysis, and displacement development
1941 (19)	Martin, A. J. P., and Synge, R. L. M.	Presented first model that could describe column efficiency; developed liquid–liquid chromatography; received Nobel Prize in 1952
1944 (20)	Consden, R., Gordon, A. H., and Martin, A. J. P.	Developed paper chromatography

TABLE 1.1 (*Continued*)

Year (Reference)	Scientist(s)	Comments
1946 (21)	Claesson, S.	Developed liquid–solid chromatography with frontal and displacement development analysis; coworker A. Tiselius
1949 (22)	Martin, A. J. P.	Contributed to relationship between retention and thermodynamic equilibrium constant
1951 (23)	Cremer, E.	Introduced gas–solid chromatography
1952 (24)	Phillips, C. S. G.	Developed liquid–liquid chromatography by frontal technique
1952 (25)	James, A. T., and Martin, A. J. P.	Introduced gas–liquid chromatography
1955 (26)	Glueckauf, E.	Derived first comprehensive equation for the relationship between HEPT and particle size, particle diffusion, and film diffusion ion exchange
1956 (27)	van Deemter, J. J., et al.	Developed rate theory by simplifying work of Lapidus and Ammundson to Gaussian distribution function
1957 (28)	Golay, M.	Reported the development of open tubular columns
1965 (29)	Giddings, J. C.	Reviewed and extended early theories of chromatography

1.2 DEFINITIONS AND NOMENCLATURE

The definitions given in this section are a combination of those used widely and those recommended by the International Union of Pure and Applied Chemistry (IUPAC) (30). The recommended IUPAC symbol appears in parentheses if it differs from the widely used symbol.

Adjusted Retention Time t'_R. The solute total elution time minus the retention time for an unretained peak (holdup time):

$$t'_R = t_R - t_M$$

Adjusted Retention Volume V'_R. The solute total elution volume minus the retention volume for an unretained peak (holdup volume):

$$V'_R = V_R - V_M$$

Adsorbent. An active granular solid used as the column packing or a wall coating in gas–solid chromatography that retains sample components by adsorptive forces.

Adsorption Chromatography. This term is synonymous with gas–solid chromatography.

Adsorption Column. A column used in gas–solid chromatography, consisting of an active granular solid and a metal or glass column.

Air Peak. The air peak results from a sample component nonretained by the column. This peak can be used to measure the time necessary for the carrier gas to travel from the point of injection to the detector.

Absolute Temperature K. The temperature stated in terms of the Kelvin scale:

$$\mathrm{K} = {}^{\circ}\mathrm{C} + 273.15^{\circ}$$

$$0^{\circ}\mathrm{C} = 273.15\ \mathrm{K}$$

Analysis Time t_{ne}. The minimum time required for a separation:

$$t_{\mathrm{ne}} = 16R_{\mathrm{s}}^2 \frac{H}{\overline{u}} \left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1+k)^3}{k^2}$$

Area Normalization (*Raw Area Normalization*). The peak areas of each peak are summed; each peak area is then expressed as a percentage of the total:

$$A_1 + A_2 + A_3 + A_4 = \Sigma A; \qquad \%A_1 = \frac{A_1}{\Sigma A}, \text{ etc.}$$

Area Normalization with Response Factor (*ANRF*). The area percentages are corrected for the detector characteristics by determining response factors. This requires preparation and analysis of standard mixtures.

Attenuator. An electrical component made up of a series of resistances that is used to reduce the input voltage to the recorder by a particular ratio.

Band. Synonymous with zone. This is the volume occupied by the sample component during passage and separation through the column.

Band Area. Synonymous with the peak area A: the area of peak on the chromatogram.

Baseline. The portion of a detector record resulting from only eluant or carrier gas emerging from the column.

Bed Volume. Synonymous with the volume of a packed column.

Bonded Phase. A stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing. The phase may be immobilized only by in situ polymerization (crosslinking) after coating.

Capacity Factor $k(D_{\mathrm{m}})$. See *Mass distribution ratio.* (In GSC, $V_{\mathrm{A}} > V_{\mathrm{L}}$; thus smaller β values and k values occur.) This is a measure of the ability of the column to retain a sample component:

$$k = \frac{t_{\mathrm{R}} - t_{\mathrm{M}}}{t_{\mathrm{M}}}$$

Capillary Column. Synonymous with open tubular column (OTC). This column has small-diameter tubing (0.25–1.0 mm i.d.) in which the inner walls are used to support the stationary phase (liquid or solid).

Carrier Gas. Synonymous with mobile or moving phase. This is the phase that transports the sample through the column.

Chromatogram. A plot of the detector response (which uses effluent concentration or another quantity used to measure the sample component) versus effluent volume or time.

Chromatograph (*Verb*). A transitive verb meaning to separate sample components by chromatography.

Chromatograph (*Noun*). The specific instrument employed to carry out a chromatographic separation.

Chromatography. A physical method of separation of sample components in which these components distribute themselves between two phases, one stationary and the other mobile. The stationary phase may be a solid or a liquid supported on a solid.

Column. A metal, plastic, or glass tube packed or internally coated with the column material through which the sample components and mobile phase (carrier-gas) flow and in which the chromatographic separation takes place.

Column Bleed. The loss of liquid phase that coats the support or walls within the column.

Column Efficiency N. See *Theoretical plate number*.

Column Material. The material in the column used to effect the separation. An adsorbent is used in adsorption chromatography; in partition chromatography, the material is a stationary phase distributed over an inert support or coated on the inner walls of the column.

Column Oven. A thermostatted section of the chromatographic system containing the column, the temperature of which can be varied over a wide range.

Column Volume V_c. The total volume of column that contains the stationary phase. [The IUPAC recommends the column dimensions be given as the inner diameter (i.d.) and the height or length L of the column occupied by the stationary phase under the specific chromatographic conditions.] Dimensions should be given in meters, millimeters, feet, or centimeters.

Component. A compound in the sample mixture.

Concentration Distribution Ratio D_c. The ratio of the analytical concentration of a component in the stationary phase to its analytical concentration in the mobile phase:

$$D_c = \frac{\text{Amount component/mL stationary phase}}{\text{Amount component/mL mobile phase}} = \frac{C_S}{C_M}$$

Corrected Retention Time t_R^0. The total retention time corrected for pressure gradient across the column:

$$t_R^0 = j t_R$$

Corrected Retention Volume V_R^0. The total retention volume corrected for the pressure gradient across the column:

$$V_R^0 = j V_R$$

Cross-Sectional Area of Column. The cross-sectional area of the empty tube:

$$A_c = r_c^2 \pi = \frac{d_c^2}{4} \pi$$

Dead Time t_M. See *Holdup time.*

Dead Volume V_M. See *Holdup volume.* This is the volume between the injection point and the detection point, minus the column volume V_c. This is the volume needed to transport an unretained component through the column.

Derivatization. Components with active groups such as hydroxyl, amine, carboxyl, and olefin can be identified by a combination of chemical reactions and GC. For example, the sample can be shaken with bromine water and then chromatographed. Peaks due to olefinic compounds will have disappeared. Similarly, potassium borohydride reacts with carbonyl compounds to form the corresponding alcohols. Comparison of before and after chromatograms will show that one or more peaks have vanished whereas others have appeared somewhere else on the chromatogram. Compounds are often derivatized to make them more volatile or less polar (e.g., by silylation, acetylation, methylation) and consequently suitable for analysis by GC.

Detection. A process by which a chromatographic band is recognized.

Detector. A device that signals the presence of a component eluted from a chromatographic column.

Detector Linearity. The concentration range over which the detector response is linear. Over its linear range the response factor of a detector (peak area units per weight of sample) is constant. The linear range is characteristic of the detector.

Detector Minimum Detectable Level (*MDL*). The sample level, usually given in weight units, at which the signal-to-noise (S/N) ratio is 2.

Detector Response. The detector signal produced by the sample. It varies with the nature of the sample.

Detector Selectivity. A selective detector responds only to certain types of compound [FID, NPD, ECD, PID, etc. (see acronym definitions in Appendix B)]. The thermal conductivity detector is universal in response.

Detector Sensitivity. Detector sensitivity is the slope of the detector response for a number of sample sizes. A detector may be sensitive to either flow or mass.

Detector Volume. The volume of carrier gas (mobile phase) required to fill the detector at the operating temperature.

Differential Detector. This detector responds to the instantaneous difference in composition between the column effluent and the carrier gas (mobile phase).

Direct Injection. A term used for the introduction of samples directly onto open tubular columns (OTCs) through a flash vaporizer without splitting (should not be confused with on-column injection).

Discrimination Effect. This occurs with the split injection technique for capillary columns. It refers to a problem encountered in quantification with split injection onto capillary columns in which a nonrepresentative sample goes onto the capillary column as a result of the difference in rate of vaporization of the components in the mixture from the needle.

Displacement Chromatography. An elution procedure in which the eluant contains a compound more effectively retained than the components of the sample under examination.

Distribution Coefficient D_g. The amount of a component in a specified amount of stationary phase, or in an amount of stationary phase of specified surface area, divided by the analytical concentration in the mobile phase. The distribution coefficient in adsorption chromatography with adsorbents of unknown surface area is expressed as

$$D_g = \frac{\text{Amount component/g dry stationary phase}}{\text{Amount component/mL mobile phase}}$$

The distribution coefficient in adsorption chromatography with well-characterized adsorbent of known surface area is expressed as

$$D_s = \frac{\text{Amount component/m}^2 \text{ surface}}{\text{Amount component/mL mobile phase}}$$

The distribution coefficient when it is not practicable to determine the weight of the solid phase is expressed as

$$D_v = \frac{\text{Amount component stationary phase/mL bed volume}}{\text{Amount component/mL mobile phase}}$$

Distribution Constant $K(K_D)$. The ratio of the concentration of a sample component in a single definite form in the stationary phase to its concentration in the mobile phase. IUPAC recommends this term rather than the partition coefficient:

$$K = \frac{C_S}{C_G}$$

Efficiency of Column. This is usually measured by column theoretical plate number. It relates to peak sharpness or column performance.

Effective Theoretical Plate Number $N_{eff}(N)$. A number relating to column performance when resolution R_S is taken into account:

$$N_{eff} = \frac{16R_S^2}{(1-\alpha)^2} = 16\left(\frac{t'_R}{w}\right)^2$$

Effective plate number is related to theoretical plate number by

$$N_{\text{eff}} = N\left(\frac{k}{k+1}\right)^2$$

Electron-Capture Detector (*ECD*). A detector utilizing low-energy electrons (furnished by a tritium or ^{63}Ni source) that ionize the carrier gas (usually argon) and collect the free electrons produced. An electron-capturing solute will capture these electrons and cause a decrease in the detector current.

Eluant. The gas (mobile phase) used to effect a separation by elution.

Elution. The process of transporting a sample component through and out of the column by use of the carrier gas (mobile phase).

Elution Chromatography. A chromatographic separation in which an eluant is passed through a column during or after injection of a sample.

External Standardization Technique (*EST*). This method requires the preparation of calibration standards. The standard and the sample are run as separate injections at different times. The calibrating standard contains only the materials (components) to be analyzed. An accurately measured amount of this standard is injected. *Calculation steps for standard:* (1) for each peak to be calculated, calculate the amount of component injected from the volume injected and the known composition of the standard; then (2) divide the peak area by the corresponding component weight to obtain the absolute response factor (ARF):

$$\text{ARF} = \frac{A_1}{W_1}$$

Calculation Step for Sample. For each peak, divide the measured area by the absolute response factor to obtain the absolute amount of that component injected:

$$\frac{A_1}{\text{ARF}} = W_i$$

Filament Element. A fine tungsten or similar wire that is used as the variable-resistance sensing element in the thermal conductivity cell chamber.

Flame Ionization Detector (*FID*). This detector utilizes the increased current at a collector electrode obtained from the burning of a sample component from the column effluent in a hydrogen and airjet flame.

Flame Photometric Detector (*FPD*). A flame ionization detector (utilizing a hydrogen-rich flame) that is monitored by a photocell. It can be specific for halogen-, sulfur-, or phosphorous-containing compounds.

Flash Vaporizer. A device used in GC where the liquid sample is introduced into the carrier-gas stream with simultaneous evaporation and mixing with the carrier gas prior to entering the column.

Flow Controller. A device used to regulate flow of the mobile phase through the column.

Flow Programming. In this procedure the rate of flow of the mobile phase is systematically increased during a part or all of the separation of higher boiling components.

Flowrate F_c. The volumetric flowrate of the mobile phase, in milliliters per minute, is measured at the column temperature and outlet pressure:

$$F_c = \frac{\pi r^2 L}{t_M}$$

Frontal Chromatography. A type of chromatographic separation in which the sample is fed continuously onto the column.

Fronting. Asymmetry of a peak such that, relative to the baseline, the front of the peak is less sharp than the rear portion.

Gas Chromatograph. A collective noun for those chromatographic modules of equipment in which gas chromatographic separations can be realized.

Gas Chromatography (*GC*). A collective noun for those chromatographic methods in which the moving phase is a gas.

Gas–Liquid Chromatography (*GLC*). A chromatographic method in which the stationary phase is a liquid distributed on an inert support or coated on the column wall and the mobile phase is a gas. The separation occurs by the partitioning (differences in solubilities) of the sample components between the two phases.

Gas-Sampling Valve. A bypass injector permitting the introduction of a gaseous sample of a given volume into a gas chromatograph.

Gas–Solid Chromatography (*GSC*). A chromatographic method in which the stationary phase is an active granular solid (adsorbent). The separation is performed by selective adsorption on an active solid.

Heartcutting. This technique utilizes a precolumn (usually packed) and a capillary column. With this technique only the region of interest is transferred to the main column; all other materials are backflushed to the vent.

Height Equivalent to an Effective Plate H_{eff}. The number obtained by dividing the column length by the effective plate number:

$$H_{eff} = \frac{L}{N_{eff}}$$

Height Equivalent to a Theoretical Plate H. The number obtained by dividing the column length by the theoretical plate number:

$$H = \frac{L}{N} = \text{HETP}$$
$$= \frac{H}{d}$$

where d is the particle diameter in a packed column or the tube diameter in a capillary column.

Holdup Time t_M. The time necessary for the carrier gas to travel from the point of injection to the detector. This is characteristic of the instrument, the *mobile-phase* flowrate, and the column in use.

Holdup Volume V_M. The volume of mobile phase from the point of injection to the point of detection. In GC it is measured at the column outlet temperature and pressure and is a measure of the volume of carrier gas required to elute an unretained component (including injector and detector volumes):

$$V_M = t_M F_c$$

Initial and Final Temperatures T_1 and T_2. This temperature range is used for a separation in temperature-programmed chromatography.

Injection Point t_0. The starting point of the chromatogram, which corresponds to the point in time when the sample was introduced into the chromatographic system.

Injection Port. Consists of a closure column on one side and a septum inlet on the other through which the sample is introduced (through a syringe) into the system.

Injection Temperature. The temperature of the chromatographic system at the injection point.

Injector Volume. The volume of carrier gas (mobile phase) required to fill the injection port of the chromatograph.

Integral Detector. This detector is dependent on the total amount of a sample component passing through it.

Integrator. An electrical or mechanical device employed for a continuous summation of the detector output with respect to time. The result is a measure of the area of a chromatographic peak (band).

Internal Standard. A pure compound added to a sample in known concentration for the purpose of eliminating the need to measure the sample size in quantitative analysis and for correction of instrument variation.

Internal Standardization Technique (*IST*). A technique that combines the sample and standard into one injection. A calibration mixture is prepared containing known amounts of each component to be analyzed, plus an added compound that is not present in the analytical sample.

Calculation steps for calibration standard:

1. For each peak, divide the measured area by the amount of that component to obtain a response factor:

$$(\mathrm{RF})_1 = \frac{A_1}{W_1}, \text{ etc.}$$

2. Divide each response factor by that of the internal standard to obtain relative response factors (RRF):

$$\mathrm{RRF}_1 = \frac{(\mathrm{RF})_1}{(\mathrm{RF})_i}$$

Calculation steps for sample:

1. For each peak, divide the measured area by the proper relative response factor to obtain the corrected area:

$$(\mathrm{CA})_1 = \frac{A_1}{\mathrm{RRF}_1}$$

2. Divide each corrected area by that of the internal standard to obtain the amount of each component relative to the internal standard:

$$(\mathrm{RW})_1 = \frac{(\mathrm{CA})_1}{(\mathrm{CA})_i}$$

3. Multiply each relative amount by the actual amount of the internal standard to obtain the actual amounts of each component:

$$(\mathrm{RW})_1 W_i = W_1$$

Interstitial Fraction $\varepsilon_\perp$. The interstitial volume per unit of packed column:

$$\varepsilon_\mathrm{I} = \frac{V_\mathrm{I}}{X}$$

Interstitial Velocity of Carrier Gas u. The linear velocity of the carrier gas inside a packed column calculated as the average over the entire cross section. Under idealized conditions it can be calculated as

$$u = F_\mathrm{c}\varepsilon_\mathrm{I}$$

Interstitial Volume $V_\mathrm{G}(V_\mathrm{I})$. The volume occupied by the mobile phase (carrier gas) in a packed column. This volume does not include the volumes external to the packed section, that is, the volume of the sample injector and the volume of the detector. In GC it corresponds to the volume that would be occupied by the carrier gas at atmospheric pressure and zero flowrate in the packed section of the column.

Ionization Detector. A chromatographic detector in which the sample measurement is derived from the current produced by the ionization of sample molecules. This ionization may be induced by thermal, radioactive, or other excitation sources.

Isothermal Mode. A condition wherein the column oven is maintained at a constant temperature during the separation process.

Katharometer. This term is synonymous with the term *thermal conductivity cell*; it is sometimes spelled “catharometer.”

Linear Flowrate F_c. The volumetric flowrate of the carrier gas (mobile phase) measured at column outlet and corrected to column temperature; and F_a is volumetric flowrate measured at column outlet and ambient temperature:

$$F_c = F_a \left(\frac{T_c}{T_a}\right) \frac{P_a - P_w}{P_a}$$

where T_c is column temperature (K), T_a is ambient temperature (K), P_a is ambient pressure, and P_w is partial pressure of water at ambient temperature.

Linear Velocity u. The linear flowrate F_c, divided by the cross-sectional area of the column tubing available to the mobile phase:

$$u = \frac{F_c}{A_c} = \frac{F_c}{r_c^2 \pi} = \frac{L}{t_M}$$

where A_c is the cross-sectional area of the column tubing, r_c is the tubing radius, and π is a constant. The equation given above is applicable for capillary columns but not for packed columns; for packed columns, the equation becomes

$$u = \frac{F_c}{\varepsilon_I r_c^2 \pi}$$

Thus, one must account for the interstitial fraction of the packed column.

Liquid Phase. Synonymous with stationary phase or liquid substrate. It is a relatively nonvolatile liquid (at operating conditions) that is either sorbed on the solid support or coated on the walls of OTCs, where it acts as a solvent for the sample. The separation results from differences in solubility of the various sample components.

Liquid Substrate. Synonymous with stationary phase.

Marker. A reference component that is chromatographed with the sample to aid in the measurement of holdup time or volume for the identification of sample components.

Mass Distribution Ratio $k(D_m)$. The fraction $(1 - R)$ of a component in the stationary phase divided by the fraction R in the mobile phase. The IUPAC recommends this term in preference to capacity factor k:

$$k(D_m) = \frac{1 - R}{R} = \frac{K}{\beta} = \frac{C_L V_L}{C_G V_G} = K \left(\frac{V_L}{V_G}\right)$$

Mean Interstitial Velocity of Carrier Gas $\overline{u}$. The interstitial velocity of the carrier gas multiplied by the pressure-gradient correction factor:

$$\overline{u} = \frac{F_c j}{\varepsilon_I}$$

Mobile Phase. Synonymous with carrier gas or gas phase.

Moving Phase. See *Mobile phase.*

Net Retention Volume V_N. The adjusted retention volume multiplied by the pressure gradient correction factor:

$$V_N = jV_R'$$

Nitrogen–Phosphorus Detector (*NPD*). This detector is selective for monitoring nitrogen or phosphorus.

On-column Injection. Refers to the method wherein the syringe needle is inserted directly into the column and the sample is deposited within the column walls rather than a flash evaporator. On-column injection differs from direct injection in that the sample is usually introduced directly onto the column without passing through a heated zone. The column temperature is usually reduced, although not as low as with splitless injections ("cool" on-column injections).

Open Tubular Column (*OTC*). Synonymous with capillary column.

Packed Column. A column packed with either a solid adsorbent or solid support coated with a liquid phase.

Packing Material. An active granular solid or stationary phase plus solid support that is in the column. The term "packing material" refers to the conditions existing when the chromatographic separation is started, whereas the term "stationary phase" refers to the conditions during the chromatographic separation.

Partition Chromatography. Synonymous with gas–liquid chromatography.

Partition Coefficient. Synonymous with the distribution constant.

Peak. The portion of a differential chromatogram recording the detector response or eluate concentration when a compound emerges from the column. If the separation is incomplete, two or more components may appear as one peak (unresolved peak).

Peak Area. Synonymous with band area. The area enclosed between the peak and peak base.

Peak Base. In differential chromatography, this is the baseline between the base extremities of the peak.

Peak Height h. The distance between the peak (band) maximum and the peak base, measured in a direction parallel to the detector response axis and perpendicular to the time axis.

Peak Maximum. The point of maximum detector response when a sample component elutes from the chromatographic column.

Peak Resolution R_S. The separation of two peaks in terms of their average peak widths:

$$R_S = \frac{2\Delta t_R}{w_a + w_b} = \frac{2\Delta t_R'}{w_a + w_b}$$

Peak Width w_b. The bar segment of the peak base intercepted by tangents to the inflection points on either side of the peak and projected on to the axis representing time or volume.

Peak Width at Half-Height w_h. The length of the line parallel to the peak base, which bisects the peak height and terminates at the intersections with the two limbs of the peak, projected onto the axis representing time or volume.

Performance Index (*PI*). This is used with open tubular columns; it is a number (in poise) that provides a relationship between elution time of a component and pressure drop. It is expressed as

$$\mathrm{PI} = 30.7H^2 \left(\frac{u}{K}\right) \frac{1+k}{k+\frac{1}{16}}$$

Phase Ratio β. The ratio of the volume of the mobile phase to the stationary phase on a partition column:

$$\beta = \frac{V_I}{V_S} = \frac{V_G}{V_A} = \frac{V_0}{V_S}$$

Photoionization Detector (*PID*). A detector in which detector photons of suitable energy cause complete ionization of solutes in the inert mobile phase. Ultraviolet radiation is the most common source of these photons. Ionization of the solute produces an increase in current from the detector, and this is amplified and passed onto the recorder.

PLOT. An acronym for *porous-layer open tubular column*, which is an open tubular column with fine layers of some adsorbent deposited on the inside wall. This type of column has a larger surface area than does a wall-coated open tubular column (WCOT).

Polarity. Sample components are classified according to their polarity (measuring in a certain way the affinity of compounds for liquid phases), for example, nonpolar hydrocarbons; medium-polarity ethers, ketones, aldehydes; and polar alcohols, acids, and amines.

Potentiometric Recorder. A continuously recording device whose deflection is proportional to the voltage output of the chromatographic detector.

Precolumn Sampling (*OTC*). Synonymous to selective sampling with open tubular columns.

Pressure P. Pressure is measured in pounds per square inch at the entrance valve to the gas chromatograph [psi = pounds per square inch = lb/in.^{2}; psia = pounds per square inch absolute = ata (atmosphere absolute); psig = pounds per square inch gauged, 1 psi = 0.069 bar].

Pressure Gradient Correction Coefficient j. This factor corrects for the compressibility of the mobile phase in a homogeneously filled column of uniform diameter:

$$j = \frac{3}{2}\left[\frac{(p_i/p_0)^2 - 1}{(p_i/p_0)^3 - 1}\right]$$

Programmed-Temperature Chromatography. A procedure in which the temperature of the column is changed systematically during a part or the whole of the separation.

Purged Splitless Injection. This term is given to a splitless injection (see *Splitless injection*) wherein the vent is open to allow the large volume of carrier gas to pass through the injector to remove any volatile materials that may be left on the column. Most splitless injections are purged splitless injections.

Pyrogram. The chromatogram resulting from sensing of the fragments of a pyrolyzed sample.

Pyrolysis. A technique by which nonvolatile samples are decomposed in the inlet system and the volatile products are separated on the chromatographic column.

Pyrolysis Gas Chromatography. A process that involves the induction of molecular fragmentation to a chromatographic sample by means of heat.

Pyrometer. An instrument for measuring temperature by the change in electrical current.

Qualitative Analysis. A method of chemical identification of sample components.

Quantitative Analysis. This involves the estimation or measurement of either the concentration or the absolute weight of one or more components of the sample.

Relative Retention $r_{a/b}$. The adjusted retention volume of a substance related to that of a reference compound obtained under identical conditions:

$$\begin{aligned} r_{a/b} &= \frac{(V_g)_a}{(V_g)_b} \\ &= \frac{(V_N)_a}{(V_N)_b} \\ &= \frac{(V'_R)_a}{(V'_R)_b} \\ &\neq \frac{(V_R)_a}{(V_R)_b} \end{aligned}$$

Required Plate Number n_{ne}. The number of plates necessary for the separation of two components based on resolution R_S of 1.5:

$$n_{ne} = 16R_S^2 \left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{1+k}{k}\right)^2$$

Resolution R_S. Synonymous with peak resolution; it is an indication of the degree of separation between two peaks.

Retention Index I. A number relating the adjusted retention volume of a compound A to the adjusted retention volume of normal paraffins. Each n-paraffin is arbitrarily allotted, by definition, an index of 100 times its carbon number. The index number of component A is obtained by logarithmic interpolation:

$$I = 100N + 100\frac{[\log V'_R(A) - \log(V'_R)(N)]}{[\log V'_R(n) - \log V'_R(N)]}$$

where N and n are the smaller and larger n-paraffin, respectively, that bracket substance A.

Retention Time (*Absolute*) t_R. The amount of time that elapsed from injection of the sample to the recording of the peak maximum of the component band (peak).

Retention Volume (*Absolute*) V_R. The product of the retention time of the sample component and the volumetric flowrate of the carrier gas (mobile phase). The IUPAC recommends that it be called *total retention volume* because it is a term used when the sample is injected into a flowing stream of the mobile phase. Thus it includes any volume contributed by the sample injector and the detector.

Sample. The gas or liquid mixture injected into the chromatographic system for separation and analysis.

Sample Injector. A device used for introducing liquid or gas samples into the chromatograph. The sample is introduced directly into the carrier-gas stream (e.g., by syringe) or into a chamber temporarily isolated from the system by valves that can be changed so as to instantaneously switch the gas stream through the chamber (gas sampling valve).

SCOT. An acronym for *support-coated open tubular column*. These are capillary columns in which the liquid substrate is on a solid support that coats the walls of the capillary column.

Selective Sampling. Refers to the transportation of a portion of a mixture onto the capillary column after it has passed through another chromatographic column, either packed or open tubular.

Separation. The time elapsed between elution of two successive components, measured on the chromatogram as the distance between the recorded bands.

Separation Efficiency N/L. A measure of the "goodness" of a column. It is usually given in terms of the number of theoretical plates per column length, that is, plates per meter for open tubular columns.

Separation Factor $\alpha_{a/b}$. The ratio of the distribution ratios or coefficients for substances A and B measured under identical conditions. By convention the separation factor is usually greater than unity:

$$\alpha_{a/b} = \frac{K_{D_a}}{K_{D_b}} = \frac{D_a}{D_b} = \frac{K_a}{K_b}$$

Separation Number (n_{sep} *or SN*). The possible number of peaks between two *n*-paraffin peaks resulting from components of consecutive carbon numbers:

$$n_{sep} = \frac{(t_{R_2} - t_{R_1})}{(w_h)_1 + (w_h)_2} - 1 = SN$$

See *Trennzahl number*.

Separation Temperature. The temperature of the chromatographic column.

Septum Bleed. Refers to the detector signal created by the vaporization of small quantities of volatile materials trapped in the septum. It is greatly reduced by allowing a small quantity of carrier gas to constantly sweep by the septum to vent.

Solid Support. The solid packing material on which the liquid phase is coated and that does not contribute to the separation process.

Solute. A synonymous term for components in a sample.

Solvent. Synonymous with liquid phase (stationary phase or substrate).

Solvent Effect (*OTC*). An effect noted in splitless injections for concentrating higher boilers at the head of the column so that the peak band will reflect the efficiency of the column and not the volume of the injection port liner. For this effect to occur, the oven temperature must be close to the boiling point of the major solvent component in the system so that it condenses at the head of the column and acts as a barrier for the solute.

Solvent Efficiency α. Synonymous with separation factor.

Solvent Venting (*OTC*). Refers to the elimination of the solvent or major ingredient in a mixture by heartcutting and flushing the solvent through the vent.

Span of the Recorder. The number of millivolts required to produce a change in the deflection of the recorder pen from 0 to 100% on the chart scale.

Specific Retention Volume V_g. The net retention volume per gram of stationary phase corrected to 0°C:

$$V_g = \frac{273\ V_N}{T W_L} = \frac{j V_R'}{T W_L}$$

Specific Surface Area. The area of a solid granular adsorbent expressed as square meter per unit weight (gram) or square meter per milliliter.

Split Injection (*OTC*). The term given to the classical method of injecting samples into a capillary system wherein the sample is introduced into a flash vaporizer and the splitter reduces the amount of sample going onto the column by the use of restrictors so that the majority of the sample goes into the vent and not onto the capillary column. Typical split ratios are 100–1 and 200–1, where the lower number refers to the quantity going onto the column.

Splitless Injection (*OTC*). The term applied to a flash vaporization technique wherein the solvent is evaporated in the injection port and condenses on the head of the column. After a suitable time (usually 0.5 min), the splitter is opened and any of the remaining material in the flash vaporizer is vented. The solvent that will have condensed at the head of the column is then slowly vaporized through column temperature programming. Splitless injection is used to concentrate small quantities of solute in a large injection (2–3 μL) onto a capillary column. The solute should have a higher boiling point than the condensed solvent so that its relative retention time is at least 1.5 and its retention index is greater than 600.

Splitter. A fitting attached to the injection port or column exit to divert a portion of the flow. It is used on the inlet side to permit the introduction of very small samples to a capillary column and on the outlet side to permit introduction of a very small sample of the column effluent to the detector, to permit introduction of effluent to two detectors simultaneously or to collect part of a peak from a destructive detector.

Stationary Phase. Synonymous with liquid phase, distributed on a solid, in gas–liquid chromatography or the granular solid adsorbent in gas–solid chromatography. The liquid may be chemically bonded to the solid.

Stationary-Phase Fraction ε_S. The volume of the stationary phase per unit volume of the packed column:

$$\varepsilon_S = \frac{V_S}{X}$$

Stationary-Phase Volume $V_L(V_S)$. The total volume of stationary-phase liquid on the support material in a particular column:

$$V_L = \frac{w_L}{\text{density}_L}$$

Surface Area. The area of a solid granular adsorbent A.

Tailing. In this condition the asymmetry of a peak is such that, relative to the baseline, the front is steeper than the rear.

Temperature Programming. In this procedure the temperature of the column is changed systematically during part or all of the separation process.

Theoretical Plate Number N. This number defines the efficiency of the column or sharpness of peaks:

$$N = 16\left(\frac{\text{peak retention time}}{\text{peak width}}\right)^2$$
$$= 16\left(\frac{t_R}{w}\right)^2$$

Thermal Conductivity. A physical property of a substance, serving as an index of its ability to conduct heat from a warmer to a cooler surface.

Thermal Conductivity Detector (*TCD*). A chamber in which an electrically heated element will reflect changes in thermal conductivity within the chamber atmosphere. The measurement is possible because of the change in resistance of the element.

Thermistor Bead Element. A thermal conductivity detection device in which a small glass-coated semiconductor sphere is used as the variable resistive element in the cell chamber.

Trennzahl Number Tz. This term is comparable with separation number and is calculated from the resolution between two consecutive members of a homologous hydrocarbon series. It is usually considered as the number of peaks that could be placed between those two members of the series. It is used predominantly in capillary column work and is expressed as

$$Tz = \left[\frac{t_{R_2} - t_{R_1}}{(w_h)_1 + (w_h)_2}\right] - 1$$

True Adsorbent Volume V_A. The weight of the adsorbent packing is divided by the adsorbent density:

$$V_A = \frac{W_A}{D_A}$$

van Deemter Equation. This equation expresses the extent to which a component band spreads as it passes through the column in terms of physical constants and the velocity of the mobile phase:

$$\text{HEPT}(H) = A + \frac{B}{u} + Cu$$

where HEPT = height equivalent to a theoretical plate
u = linear velocity of carrier gas (mobile phase);
$\overline{u}$ = average linear carrier-gas velocity
A = constant that accounts for the effects of "eddy" diffusion in the column
B = constant that accounts for the effect of molecular diffusion of the vapor in the direction of the column axis
C = constant proportional to the resistance of the column packing to mass transfer of solute through it

Velocity of Mobile Phase u. Synonymous with linear velocity.

WCOT. An acronym for *wall-coated open tubular column*. It is a capillary column in which the inside wall is coated with the stationary phase.

Weight of Stationary Liquid Phase W_L. The weight of liquid phase in the column.

WWCOT. A *whisker-wall-coated open tubular column*. It is a WCOT in which the walls have been etched before the stationary phase is deposited.

WWPLOT. An acronym for whisker-wall porous-layer open tubular column. It is a PLOT column in which the walls have been etched before deposition of the support.

WWSCOT. An acronym for *whisker-wall-support-coated open tubular column*. It is a SCOT column in which the walls have been etched before depositing of the support.

Zone. The position and spread of a solute within the column, the region in the chromatographic bed where one or more components of the sample are located. See *Band.*

1.3 SUGGESTED READING ON GAS CHROMATOGRAPHY

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1.4 COMMERCIAL INSTRUMENTATION

All leading instrument manufacturers produce and market gas chromatographs. In addition, many smaller speciality companies also manufacture and market GC units. Which instrument should be considered depends on the use to which they are to be utilized, and this ultimately establishes the criteria for purchase. GC units come in a variety of makes and models, from simple student instructional types (e.g., Gow-Mac Instrument Co.) up to deluxe multicolumn, interchangeable detector types (e.g., Agilent Technologies). We refer the reader to the "Lab Guide" issue of the *Journal of Analytical Chemistry* (**31**), *American Laboratory Journal* (**32**), and *LC/GC Journal* (**33**), rather than to one particular company, for a listing of the instrument manufacturers.

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PART I

Theory and Basics

Science moves, but slowly slowly, creeping on from point to point.
—Alfred, Lord Tennyson (1809–1892)
Locksley Hall, line 134

CHAPTER TWO

Theory of Gas Chromatography

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2.2 GENERAL ASPECTS OF GAS CHROMATOGRAPHY
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2.3 GAS CHROMATOGRAPHY
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REFERENCES

2.1 CHROMATOGRAPHIC METHODS

2.1.1 Classification of Methods

In the strictest sense, the term "chromatography" is a misnomer. Most of the materials chromatographed today are either colorless, or, if they were colored, one

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would not be able to perceive them in most instances. A number of workers in the field have offered contemporary definitions of the term, but not all practitioners of the technique use these terms or even agree with them. In the paragraph that follows we present our own definition but do not declare it to be unique or more representative of the process.

Chromatography encompasses a series of techniques that have in common the separation of components of a mixture by a series of equilibrium operations that result in separation of the entities as a result of their partitioning (differential sorption) between two different phases, one stationary with a large surface and the other a moving phase in contact with the first. Chromatography is not restricted to analytical separations. It may be used in the preparation of pure substances, the study of the kinetics of reactions, structural investigations on the molecular scale, and the determination of physicochemical constants, including stability constants of complexes, enthalpy, entropy, and free energy (see Chapter 12).

Using the definition given in the preceding paragraph (or any other definition of chromatography), one can tabulate numerous variations of the technique (see Figure 2.1). Our specific concern is the gas chromatographic technique. For

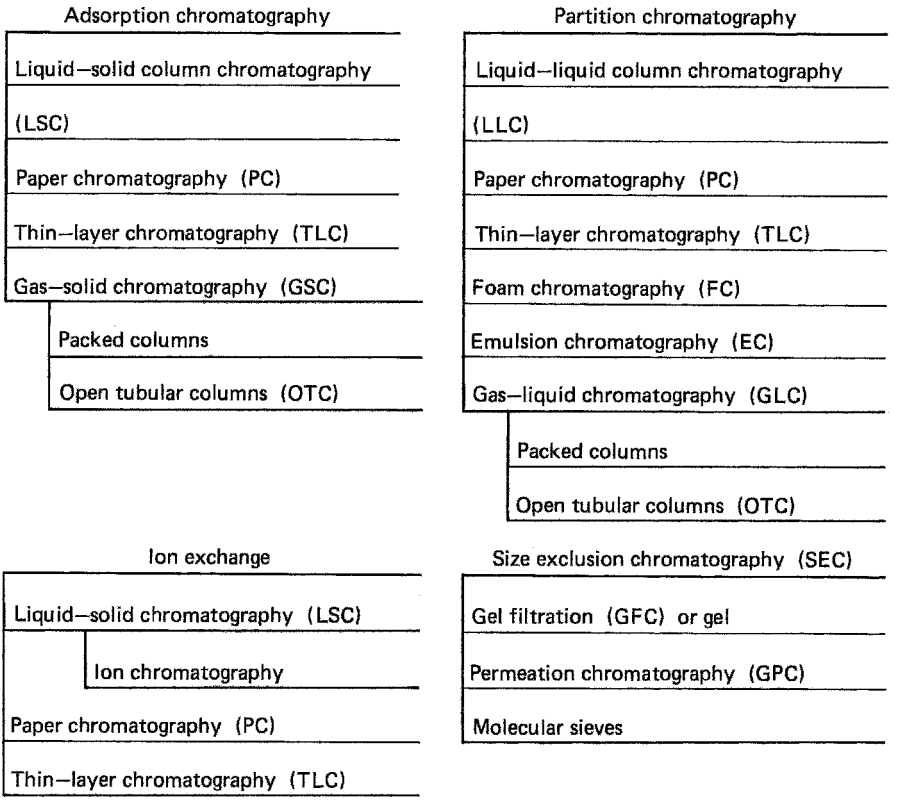

FIGURE 2.1 Various chromatographic techniques.

this technique we have available different types of column that may be used to perform the separation. More details are found in Chapters 3 and 4.

2.1.2 General Aspects

The mixture to be separated and analyzed may be either a gas, a liquid, or a solid in some instances. All that is required is that the sample components be stable, have a vapor pressure of approximately 0.1 Torr at the operating temperature, and interact with the column material (either a solid adsorbent or a liquid stationary phase) and the mobile phase (carrier gas). The result of this interaction is the differing distribution of the sample components between the two phases, resulting in the separation of the sample component into zones or bands. The principle that governs the chromatographic separation is the foundation of most physical methods of separation, for example, distillation and liquid–liquid extraction.

Separation of the sample components may be achieved by one of three techniques: frontal analysis, displacement development, or elution development.

2.1.3 Frontal Analysis

The liquid or gas mixture is fed into a column containing a solid packing. The mixture acts as its own mobile phase or carrier, and the separation depends on the ability of each component in the mixture to become a sorbate (see Figure 2.2). Once the column packing has been saturated (i.e., when it is no longer able to sorb more components), the mixture then flows through with its original composition. The early use of this technique involved measurement of the change in concentration of the front leaving the column; hence the name "frontal analysis." The least-sorbed component breaks through first and is the only component to be obtained in a pure form. Figure 2.3 illustrates the integral-type recording for this type of system. In this figure we illustrate the recording of the fronts from a four-component sample.

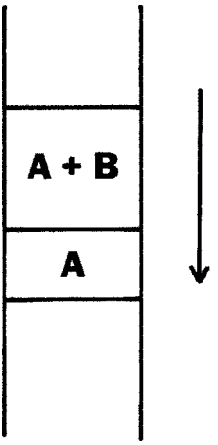

FIGURE 2.2 Frontal analysis. Component B is more sorbed than component A.

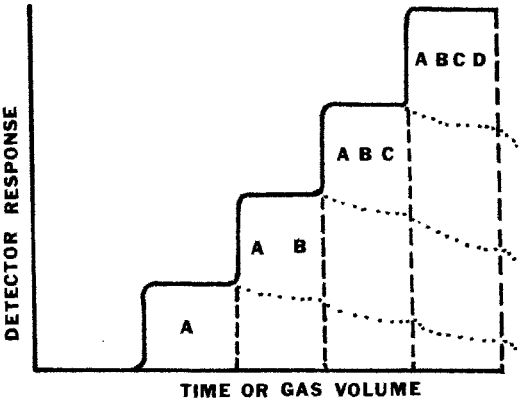

FIGURE 2.3 Integral-type chromatogram from frontal analysis. Component A is the least sorbed of four components.

Frontal analysis requires that the system have convex isotherms (see Section 2.1.6). This results in the peaks having sharp fronts and well-formed steps. An inspection of Figure 2.3 reflects the problem of analytical frontal analysis—it is difficult to calculate initial concentrations in the sample. One can, however, determine the number of components present in the sample. If the isotherms are linear, the zones may be diffuse. This may be caused by three important processes: inhomogeneity of the packing, large diffusion effects, and nonattainment of sorption equilibrium.

2.1.4 Displacement Development

In this technique the developer is contained in the moving phase, which may be a liquid or a gas (Figure 2.4). One necessary requirement is that this moving phase be more sorbed than any sample components. One always obtains a single pure band of the first component in the sample. In addition, there is always an overlap zone for each succeeding component, which is an advantage of this technique over frontal analysis. The disadvantage, from the analytical viewpoint, is that the component bands are not separated by a region of pure mobile phase. The result of this displacement mechanism (Figure 2.5) assumes a three-component mixture and a developer or displacing agent. The step height is utilized for qualitative identification of components, whereas the step length is proportional to the amount of the component.

As with frontal analysis, displacement analysis requires convex isotherms. Once equilibrium conditions have been attained, an increase in column length serves no useful purpose in this technique because the separation is more dependent on equilibrium conditions than on column size.

2.1.5 Elution Development

In this technique, components A and B travel through the column at rates determined by their retention on the solid packing (Figure 2.6). If the differences in

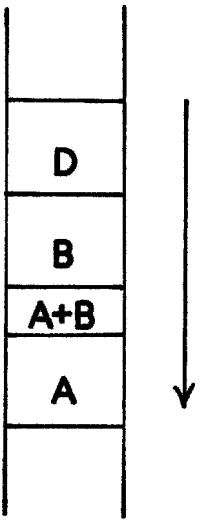

FIGURE 2.4 Displacement development (D = displacer). D is more sorbed than B, which is more sorbed than A.

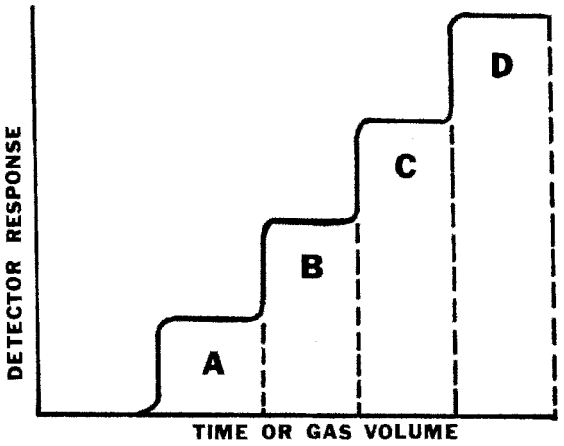

FIGURE 2.5 Integral-type chromatogram from displacement development. Order of sorption: D > C > B > A.

sorption are sufficient or the column is long enough, a complete separation of A and B is possible. Continued addition of eluant causes the emergence of separated bands or zones from the column. A disadvantage of this technique is the very long time interval required to remove a highly sorbed component. This can be overcome by increasing the column temperature during the separation process. Figure 2.7 depicts a typical chromatogram for this technique. The position of peak maximum on the abscissa qualitatively identifies the component, and the peak area is a measure of the amount of each component.

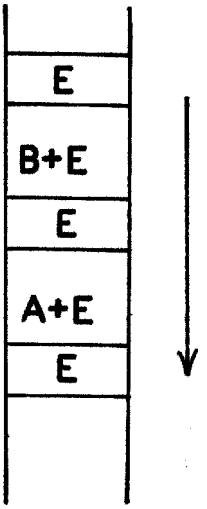

FIGURE 2.6 Elution development (E = eluant). B is more sorbed than A.

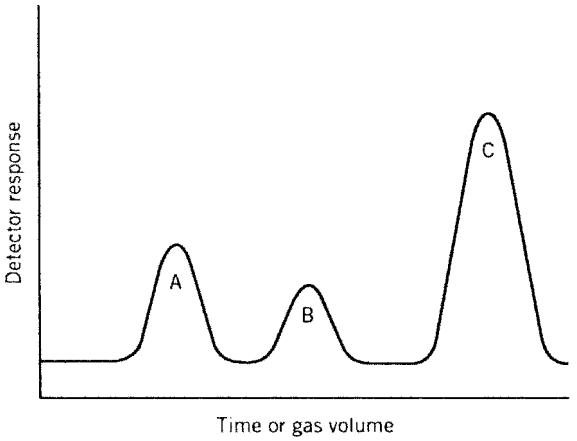

FIGURE 2.7 Differential chromatogram from elution development. Order of retention: C > B > A.

Summary

The *frontal* technique (Section 2.1.3) does not lend itself to many analytical applications because of the overlap of the bands and the requirement of a large amount of sample. However, it may be used to study phase equilibria (isotherms) and for preparative separations. (Many of the industrial chromatographic techniques use frontal analysis.) *Displacement* development (Section 2.1.4) has applications for analytical liquid chromatography (LC). (For instance, it may be used as an initial concentrating step in GC for trace analysis.) This technique may also be used in preparative work. The outstanding disadvantage of both of these techniques

is that the column still contains sample or displacer at the conclusion of the separation; thus the column must be regenerated before it can be used again.

It is in this regard that *elution* chromatography (Section 2.1.5) offers the greatest advantage—at the end of a separation, only eluant remains in the column. Thus the bulk of the discussion in the subsequent chapters is concerned with elution GC. The isotherms and chromatograms of elution chromatography are discussed in Sections 2.1.6–2.1.9.

2.1.6 Isotherms

An *isotherm* is a graphical presentation of the interaction of an adsorbent and a sorbate in solution (gas or liquid solvent) at a specified temperature. The isotherm is a graphical representation of the partition coefficient or distribution constant K:

$$K = \frac{C_S}{C_G} \tag{2.1}$$

where C_S is the concentration of sorbate in stationary phase or at the solid surface and C_G is the concentration of sorbate in the gas phase. The concentration of the substance sorbed per unit mass of sorbent is plotted against the concentration of the substance in equilibrium with the phase present at the interface. Three types of isotherm are obtainable: one linear and two curved. We describe the nonlinear isotherms as either concave (curved away from the abscissa) or convex (curved toward the abscissa). Figure 2.8 depicts these three isotherms.

The *linear isotherm* is obtained when the ratio of the concentration of substance sorbed per unit mass and concentration of the substance in solution remains constant. This means that the partition coefficient or distribution constant K (see Section 1.2) is constant over all working concentration ranges. Thus the frontal and rear boundaries of the band or zone will be symmetric.

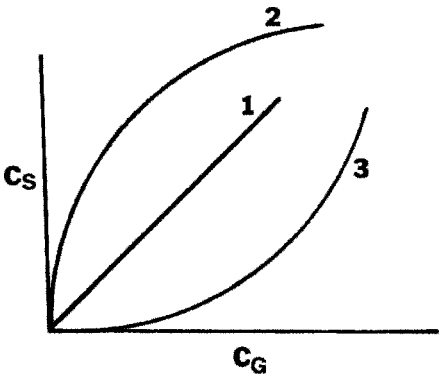

FIGURE 2.8 Isotherms: C_S = concentration at solid surface or in a stationary phase; C_G = concentration in solution at equilibrium; 1 = Linear isotherm, 2 = convex isotherm, 3 = concave isotherm.

The *convex isotherm* demonstrates that the K value is changing to a higher ratio as concentration increases. This results in movement of the component through the column at a faster rate, thus causing the front boundary to be self-sharpening and the rear boundary to be diffuse.

The *concave isotherm* results from the opposite effect (where the K value changes to a lower value), and the peak will have a diffuse front boundary and a self-sharpening rear boundary. In other words, the solute increasingly favors the surface of the stationary phase as the solution concentration increases. These effects are depicted in Figure 2.9. When the isotherms curve in either direction (convex or concave) as concentration is varied, one obtains complex chromatograms. Changing the sample concentration or physical conditions (temperature, flowrate, pressure, etc.) can help in converting the rear and front boundaries to Gaussian shape.

The most frequently applied isotherm equations are those due to Freundlich and Langmuir described in Brunauer (1).

1. *Freundlich Equation.* This equation represents the variation of adsorption with pressure over a limited range, at constant temperature:

$$\frac{x}{m} = kp^{1/n} \tag{2.2}$$

where x = mass of adsorbed gas
m = grams of adsorbing material
p = pressure
k, n = constants

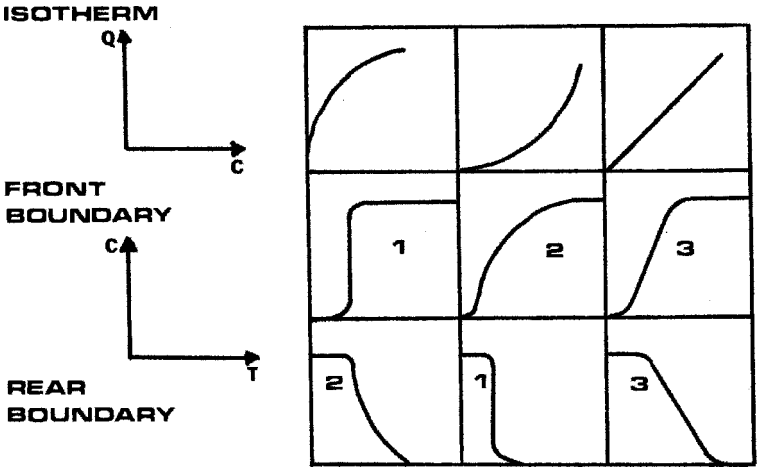

FIGURE 2.9 Dependence of boundary profile on form of partition isotherm. C = concentration (mL/mol) of solute in gas phase; Q = concentration in liquid or adsorbed phase; T = time for band to emerge from the column; 1 = self-sharpening profile, 2 = diffuse profile, 3 = Gaussian profile. (Courtesy of Wiley-Interscience Publishers).

The exponent $1/n$ is usually less than one, indicating that the amount of adsorbed gas does not increase in proportion to the pressure. If the exponent $1/n$ were unity, the Freundlich equation would be equivalent to the distribution law. Converting Equation 2.2 to log form, we obtain

$$\log \frac{x}{m} = \log k + \left(\frac{1}{n}\right) \log p \tag{2.3}$$

which is an equation of a straight line; thus the $\log x/m - \log p$ relationship is linear (linear isotherm). If a value of $1/n$ being unity gives a linear isotherm, a value of $1/n > 1$ gives a concave isotherm. When $1/n < 1$, a convex isotherm results.

2. *Langmuir Equation*. It is probable that adsorbed layers have a thickness of a single molecule because of the rapid decrease in intermolecular forces with distance. The Langmuir adsorption isotherm equation is

$$\frac{x}{m} = \frac{k_1 k_2 p}{1 + k_1 p} \tag{2.4}$$

where k_1, k_2 are constants for a given system and p is the gas pressure, which may be written as

$$\frac{p}{x/m} = \frac{1}{k_1 k_2} + \frac{p}{k_2} \tag{2.5}$$

A plot of $p(x/m)$ versus p produces a straight line with slope of $1/k_2$ and an intercept of $1/k_1 k_2$. Deviations from linearity are attributed to nonuniformity, leading to various types of adsorption on the same surface, that is, nonmonomolecular adsorption on a homogeneous surface.

2.1.7 Process Types in Chromatography

The process of chromatographic separation can be defined by two conditions:

1. The distribution isotherms (representation of the partition coefficient or distribution constant K) may be either linear or nonlinear (see Section 2.1.6).
2. The chromatographic system is either ideal *or* nonideal. *Ideal* chromatography infers that the exchange between the two phases is thermodynamically reversible. In addition, the equilibrium between the solid granular particles or liquid-coated particles and the gas phase is immediate; that is, the mass transfer is very high, and longitudinal and other diffusion processes are small enough to be ignored. In *nonideal* chromatography these assumptions cannot be made.

   Using these two sets of conditions, we can then describe four chromatographic systems: (a) linear ideal chromatography, (b) linear nonideal chromatography, (c) nonlinear ideal chromatography, and (d) nonlinear nonideal chromatography.

2.1.8 Linear Ideal Chromatography

This is the most direct and simple theory of chromatography. The transport of the solute down the column will depend on the distribution constant (partition coefficient) K and the ratio of the amounts of the two phases in the column. Band (zone) shape does not change during this movement through the column.* The system can be visualized as illustrated in Figure 2.10.

2.1.9 Linear Nonideal Chromatography

In this system the bands (zones) broaden because of diffusion effects and nonequilibrium. This broadening mechanism is fairly symmetric, and the resulting elution

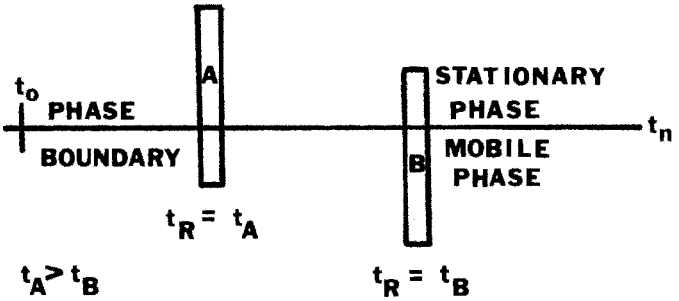

FIGURE 2.10 Linear ideal chromatography: t_0 = start of separation (point of sample injection); t_A = retention time of component A; t_B = retention time of component B; t_n = time for emergence of mobile phase from t_0.

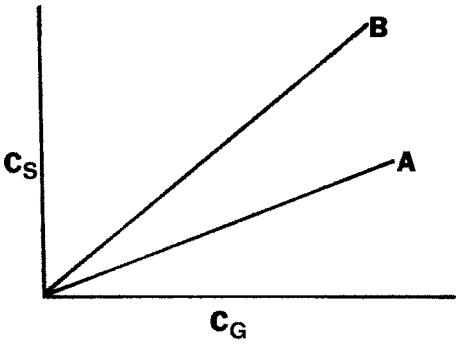

FIGURE 2.11 Isotherms for linear ideal chromatography: C_S = concentration at surface or in stationary phase; C_G = concentration in solution at equilibrium.

*This type of chromatography would be the best of all worlds—that is, there are no diffusion effects, and the mass transfer between phases is instantaneous (see Section 2.3.2). The isotherms that result from this system would be linear (see Figure 2.11).

bands approach the shape of a Gaussian curve. This system best explains liquid or gas partition chromatography. The system may be viewed in two ways:

1. *Plate Theory*. Envision the chromatographic system as a discontinuous process functioning the same as a distillation or extraction system, that is, one consisting of a large number of equivalent plates.
2. *Rate Theory*. Consider the chromatographic system as a continuous medium where one accounts for mass transfer and diffusion phenomena.

These two points of view usually are used to discuss gas chromatographic theory. Linear nonideal chromatography may be visualized by the relationships shown in Figures 2.12 and 2.13.

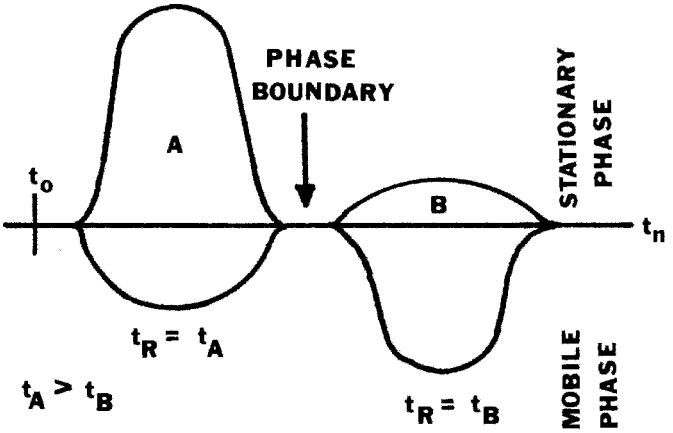

FIGURE 2.12 Linear nonideal chromatography: t_0 = time at start of separation (point of sample injection); t_A = retention time of component A; t_B = retention time of component B; t_n = time for emergence of mobile phase from t_0.

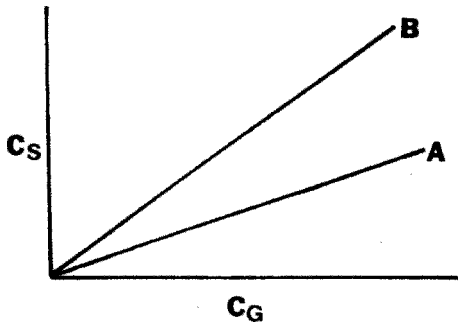

FIGURE 2.13 Isotherms for linear nonideal chromatography: C_S = concentration at surface or in stationary phase; C_G = concentration in solution (mobile phase) at equilibrium.

2.1.10 Nonlinear Ideal Chromatography

Liquid–solid chromatography is representative of this system type because nonlinearity effects are usually appreciable. Mass transfer is fast, and longitudinal diffusion effects may be ignored in describing the system. The net result is that the bands (zones) develop self-sharpening fronts and diffuse rear boundaries. Because of this tailing, this technique is unsuitable for elution analysis. This system is represented by Figures 2.14 and 2.15.

2.1.11 Nonlinear Nonideal Chromatography

Gas–solid chromatography is best described by this theory. Here one finds diffuse front and rear boundaries with definite tailing of the rear boundary. Mathematical descriptions of systems of this type can become very complex; however, with proper assumptions mathematical treatments do fairly represent the experimental data. The bands (zones) are similar to those shown in Figures 2.16 and 2.17.

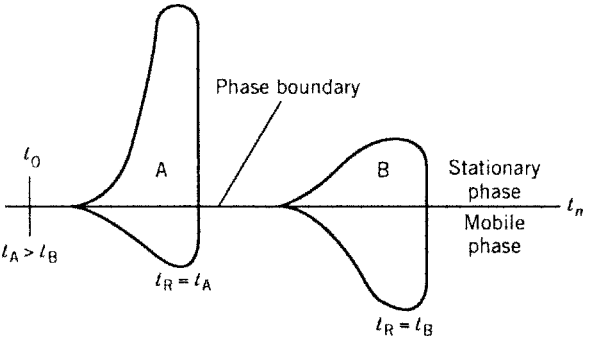

FIGURE 2.14 Nonlinear ideal chromatography: t_0 = start of separation (point of sample injection); t_A = retention time of component A; t_B = retention time of component B; t_n = time of emergence of mobile phase from t_0.

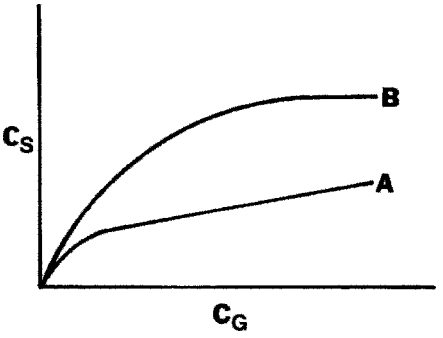

FIGURE 2.15 Isotherms for nonlinear ideal chromatography: C_S = concentration at surface or in stationary phase; C_G = concentration in solution (mobile phase) at equilibrium.

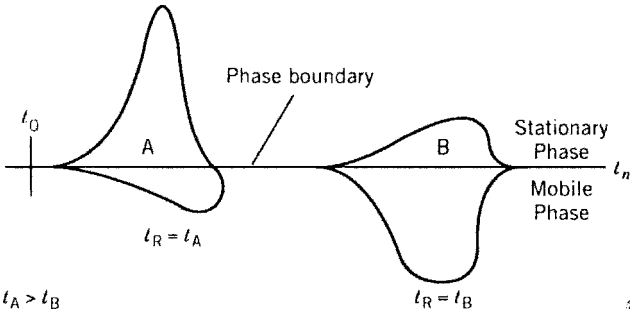

FIGURE 2.16 Nonlinear nonideal chromatography: t_0 = start of separation (point of sample injection); t_A = retention time of component A; t_B = retention time of component B; t_n = time of emergence of mobile phase from t_0.

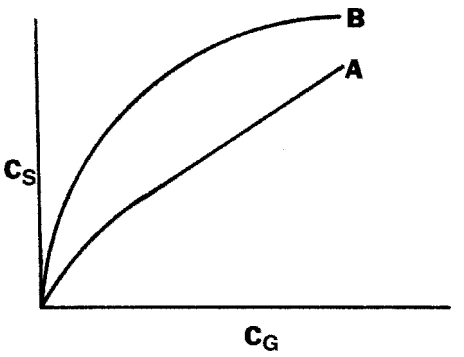

FIGURE 2.17 Isotherms for nonlinear nonideal chromatography. C_S = concentration at surface or in stationary phase; C_G = concentration in solution (mobile phase) at equilibrium.

2.2 GENERAL ASPECTS OF GAS CHROMATOGRAPHY

2.2.1 Applications of Gas Chromatography

Gas chromatography is a unique and versatile technique. In its initial stages of development it was applied to the analysis of gases and vapors from very volatile components. The work of Martin and Synge (2) and then James and Martin (3) in gas–liquid chromatography (GLC) opened the door for an analytical technique that has revolutionized chemical separations and analyses. As an analytical tool, GC can be used for the direct separation and analysis of gaseous samples, liquid solutions, and volatile solids.

If the sample to be analyzed is nonvolatile, the techniques of derivatization or pyrolysis GC can be utilized. This latter technique is a modification wherein a nonvolatile sample is pyrolyzed before it enters the column. Decomposition products are separated in the gas chromatographic column, after which they are qualitatively and quantitatively determined. Analytical results are obtained

from the *pyrogram* (a chromatogram resulting from the detection of pyrolysis products). This technique can be compared to mass spectrometry, a technique in which analysis is based on the nature and distribution of molecular fragments that result from the bombardment of the sample component with high-speed electrons. In pyrolysis GC the fragments result from chemical decomposition by heat. If the component to be pyrolyzed is very complex, complete identification of all the fragments may not be possible. In a case of this type, the resulting pyrogram may be used as a set of "fingerprints" for subsequent study.

Pyrolysis may be defined as the thermal transformation of a compound (single entity) into another compound or compounds, usually in the absence of oxygen. In modern pyrolysis the sample decomposition is rigidly controlled. One should keep in mind that pyrolysis gas chromatography (PGC) is an indirect method of analysis in which heat is used to change a compound into a series of volatile products that should be characteristic of the original compound and the experimental conditions.

Gas chromatography is the analytical technique used for product identification (under very controlled conditions) and must be directly coupled to a mass spectrometer when information other than a comparative fingerprint (pyrogram) is required, such as positive identification of peaks on the chromatogram.

Ettre and Zlatkis (6) classified pyrolysis types according to extent of degradation of the sample compound:

1. *Thermal Degradation*. Usually occurs in the temperature range of 100–300°C but may occur as high as 500°C. This type may be carried out in the injection port of the instrument. Rupture of carbon–carbon bonds is minimal.
2. *Mild Pyrolysis*. Occurs between 300 and 500°C, and carbon–carbon bond breakage occurs to some extent.
3. *Normal Pyrolysis*. Occurs between 500 and 800°C and involves cleavage of carbon–carbon bonds. Very useful for characterizing polymers and copolymers.
4. *Vigorous Pyrolysis*. Occurs at temperatures between 800 and 1100°C. The end results is the breaking of carbon–carbon bonds and cleaving organic molecules into smaller fragments.

The pyrolysis process may be performed by three different methods:

1. *Continuous-Mode Method*. May involve tube furnaces or microreactors. In this mode the heated wall of the reactor is at a higher temperature than the sample and secondary reactions of pyrolysis products will most likely occur.
2. *Pulse-Mode Pyrolysis*. Sample is in direct contact with a hot wire, thus minimizing secondary reactions. Although the temperature profile is reproducible, the exact pyrolysis temperature cannot be measured. Another disadvantage is that the sample weight cannot be known accurately. This is also known as *Curie point pyrolysis*.

3. *Laser-Mode Pyrolysis*. Directs very high energies to the sample, which usually result in ionization and the formation of plasma plumes. Thus, laser pyrolysis results in fewer and sometimes different products than thermal pyrolysis.

To a first approximation, good interlaboratory reproducibility of the pyrolysis profile is obtainable; however, intralaboratory matchings have been disappointing. Several major parameters influence pyrolysis reproducibility:

1. Type of pyrolyzer
2. Temperature
3. Sample size and homogeneity
4. Gas chromatographic conditions and column(s) used
5. Interface between the pyrolyzer and the gas chromatograph.

Therefore, optimization of the pyrolyzer by use of reference standards is important. Thermal gradients across the sample may be avoided by use of thin samples. For good results in PGC, one must have rapid transfer of the pyrolysis products to the column, minimization of secondary reaction products, and elimination of poor sample injection profiles.

When employing PGC for qualitative and quantitative analysis of complex unknown samples, it is essential to use *pure* samples of suspected sample components as a reference. One should never base identification of unknown pyrolyzate peaks on the retention time of pyrolyzate product peaks obtained from the standard (7). A peak in the chromatogram from the pyrolysis of the unknown may be from the matrix and not the suspected component. The use of selective detectors (i.e., a NPD with a FID or a FID with an ECD) will furnish element information but not molecular or structural information about the component peak. The matrix components (in the absence of the suspected analyte) may yield the same peak at the same retention time.

Another important variable in PGC is temperature control. Small changes in temperature may have pronounced effects on the resulting chromatogram. The effects may be manifested in several ways:

1. Increased number of peaks
2. Decreased number of peaks
3. Partial resolution of overlapping peaks
4. Increase or decrease in the peak areas for same sample size of unknown (indicating different pyrolysis mechanism)
5. Changes in peak shape of pyrolysis products

Thus, caution must be used when identifying a peak on a pyrogram for an unknown. This means that a reliable identification should not be based on retention time data. The two best techniques for identifying unknown peaks are

infrared spectroscopy (IRS) and mass spectrometry (MS). Mass spectrometry is the better of the two techniques because one obtains a mass number that may be matched with a mass number in a library of mass spectra of known compounds. *All* the ions from a known compound must be present for positive identification. Infrared spectroscopy will validate the presence of functional groups in the molecule. If the peak is single entity, one may match the spectrum (IR) obtained with a spectrum of a standard compound.

In addition to analysis, GC may be used to study structure of chemical compounds, determine the mechanisms and kinetics of chemical reactions, and measure isotherms, heats of solution, heats of adsorption, free energy of solution and/or adsorption, activity coefficients, and diffusion constants (see Chapter 12). Another significant application of GC is in the area of the preparation of pure substances or narrow fractions as standards for further investigations. Gas chromatography is also utilized on an industrial scale for process monitoring. In adsorption studies it can be used to determine specific surface areas (4,5). A novel use is its utilization for elemental analyses of organic components (8–10). Distillation curves may also be plotted from gas chromatographic data.

Gas chromatography can be applied to the solution of many problems in various fields. A few examples are enumerated:

1. *Drugs and Pharmaceuticals*. Gas chromatography is used not only in the quality control of products of this field but also in the analysis of new products and the monitoring of metabolites in biological systems.

2. *Environmental Studies*. A review of the contemporary field of air pollution analyses by GC was published in the first volume of *Contemporary Topics in Analytical and Clinical Chemistry* (11). A book by Grob and Kaiser (12) discussed the use of LC and GC for this type of analysis. Many chronic respiratory diseases (asthma, lung cancer, emphysema, and bronchitis) could result from air pollution or be directly influenced by air pollution. Air samples can be very complex mixtures, and GC is easily adapted to the separation and analysis of such mixtures. Two publications concerned with the adaptation of cryogenic GC to analyses of air samples are References 13 and 14. Chapter 15 covers the application of GC in the environmental area.

3. *Petroleum Industry*. The petroleum companies were among the first to make widespread use of GC. The technique was successfully used to separate and determine the many components in petroleum products. One of the earlier publications concerning the response of thermal conductivity detectors to concentration resulted from research in the petroleum field (15). The application of GC to the petroleum field is discussed in Chapter 13.

4. *Clinical Chemistry*. Gas chromatography is adaptable to such samples as blood, urine, and other biological fluids (see Chapter 14). Compounds such as proteins, carbohydrates, amino acids, fatty acids, steroids, triglycerides, vitamins, and barbiturates are handled by this technique directly or after preparation of appropriate volatile derivatives (see Chapter 14).

5. *Pesticides and Their Residues*. Gas chromatography in combination with selective detectors such as electron capture, phosphorus, and electrolytic conductivity detectors (see Chapter 6) have made the detection of such components and their measurement relatively simple. Detailed information in this area may be found in a monograph by Grob (16) and Chapter 15 of this book.

6. *Foods*. The determination of antioxidants and food preservatives is an active part of the gas chromatographic field. Adaptations and sample types are almost limitless, and include analysis of fruit juices, wines, beers, syrups, cheeses, beverages, food aromas, oils, dairy products, decomposition products, contaminants, and adulterants.

2.2.2 Types of Detection

The various detectors employed in GC are discussed in Chapter 6. Our purpose here is only to categorize the detection system according to whether they are an integral-type system or a differential-type system. This classification is an old one; any detection system can be made integral or differential simply by a modification of the detector electronics. A more modern categorization would be *instantaneous* (differential) and *cumulative* (integral). Chromatograms that result from this classification of detectors are shown in Figure 2.18.

2.2.3 Advantages and Limitations

From the limited discussion so far one can visualize the versatility of the gas chromatographic technique. There are so many reasons for this, and we shall

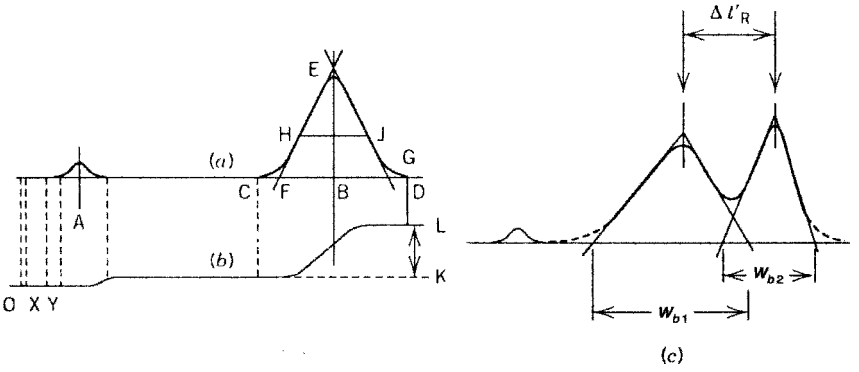

FIGURE 2.18 Types of chromatogram: *a*, differential chromatogram; *b*, integral chromatogram; *c*, peak resolution; *O*, injection point; *OX*, injector volume; *OY*, detector volume; *OA*, holdup volume V_M; *OB*, total retention volume V_R; *AB*, adjusted retention volume $V_R - V_M$; *CD*, peak base; *FG*, peak width w_b; *HJ*, peak width at half-height w_h; *BE*, peak height; *E*, peak maximum; *CHEJD*, peak area (space incorporated within these letters); *KL*, step height of integral chromatogram.

enumerate some of the advantages. It should be stressed that what one person considers a disadvantage may be an advantage to someone else. Additionally, a current disadvantage, may be an advantage several years from now.

A few broad comments regarding GC would include the following:

1. *An Analytical Technique*. This is used not only for the qualitative identification of components in a sample, but also for quantitative measurements.
2. *A Physical Research Technique*. This may be used to investigate various parameters of a system, such as determination of partition coefficients, thermodynamic functions, and adsorption isotherms (see Chapter 12).
3. *A Preparative Technique*. Once the analytical conditions have been determined, the system may be scaled up to separate and collect gram amounts of components.
4. *An Online Monitoring Probe*. A gas chromatograph can be locked into a process line so that the process stream may be monitored on a 24-h basis.
5. *An Automated System*. A gas chromatograph may be interfaced to a computer with an automatic sampler so that routine analyses can be run overnight.

Following are some overall advantages of GC that should be stressed:

1. *Resolution*. The technique is applicable to systems containing components with very similar boiling points. By choosing a selective liquid phase or the proper adsorbent, one can separate molecules that are very similar physically and chemically. Components that form azeotropic mixtures in ordinary distillation techniques may be separated by GC.
2. *Sensitivity*. This property of the gas chromatographic system largely accounts for its extensive use. The simplest thermal conductivity detector cells can detect a few parts per million; with an electron capture detector or phosphorous detector, parts per billion or picograms of solute can easily be measured. This level of sensitivity is more impressive when one considers that the sample size used is of the order of 1 μL or less.
3. *Analysis Time*. Separation of all the components in a sample may take from several seconds up to 30 min. Analyses that routinely take an hour or more may be reduced to a matter of minutes, because of the high diffusion rate in the gas phase and the rapid equilibrium between the moving and stationary phases(see Chapter 5).
4. *Convenience*. The operation of GC is a relatively straightforward procedure. It is not difficult to train nontechnical personnel to carry out routine separations.
5. *Costs*. Compared with many analytical instruments available today, gas chromatographs represent an excellent value.
6. *Versatility*. Gas chromatography is easily adapted for analysis of samples of permanent gases as well as high-boiling liquids or volatile solids.

7. *High Separating Power*. Since the mobile phase has a low degree of viscosity, very long columns with excellent separating power can be employed.
8. *Assortment of Sensitive Detecting Systems*. Gas chromatographic detectors (see Chapter 6) are relatively simple and highly sensitive, and possess rapid response rates.
9. *Ease of Recording Data*. Detector output from gas chromatographs can be conveniently interfaced with recording potentiometers, integrating systems, computers, and a wide variety of automatic data storing modules (see Chapter 6).
10. *Automation*. Gas chromatographs may be used to monitor automatically various chemical processes in which samples may be periodically taken and injected onto a column for separation and detection.

2.3 GAS CHROMATOGRAPHY

It was pointed out in Section 2.1 that chromatographic separations can be evaluated by the shape of the peaks from a particular system. Peak shapes depend on the isotherms that describe the relationship between concentration of solute in the stationary phase to the solute concentration in the carrier gas. If the isotherms are linear, the peaks are Gaussian in shape and the separations proceed with little or no problems. If the isotherms are nonlinear, the peaks become asymmetric. Some isotherms are linear over a limited range, and as long as we work in this limited range, few problems are encountered. If the isotherm is concave to the gas-phase concentration axis (so that the distribution ratio decreases with the increase in solute concentration in the mobile phase), the band will have a sharp front and a long tail. If, on the other hand, the isotherm is convex to the gas-phase concentration axis (so that the distribution ratio increases with increase in solute in the mobile phase), the band will have a leading front and a sharp rear edge.

If chromatographic theory is explained on the basis of a discontinuous model, several assumptions are made: (1) equilibrium between solute concentration in the two phases is reached instantaneously; (2) diffusion of solute, in the mobile phase, along the column axis is minimal; and (3) the column is packed uniformly or wall-coated uniformly. All these conditions are not present in all chromatographic separations.

If the rate constants for the sorption–desorption processes are small, equilibrium between phases need not be achieved instantaneously. This effect is often called *resistance-to-mass transfer*, and thus transport of solute from one phase to another can be assumed to be diffusional in nature. As the solute migrates through the column, it is sorbed from the mobile phase into the stationary phase. Flow is through the void volume of the solid particles, with the result that the solute molecules diffuse through the interstices to reach the surface of the stationary phase. Likewise, the solute must diffuse from the interior of the stationary phase to get back into the mobile phase.

When the term “longitudinal diffusion” is applied to the chromatographic band, we include the true longitudinal molecular diffusion (Section 2.3.2) and

apparent longitudinal diffusion or eddy diffusion. True longitudinal diffusion occurs because of concentration gradients within the mobile phase, but eddy diffusion results from uneven velocity profiles because of unequal lengths and widths of the large number of zigzag paths. As a result of these diffusion effects, some solute molecules move ahead, whereas others lag behind the center of the zone (band). The widening of the band as it moves down the column is of paramount importance in GC. The extent to which the band spreads (peak sharpness) determines the column efficiency N (theoretical plate number).

2.3.1 Plate Theory

From the equilibrium shown in Equation 11.19, it follows that

$$\alpha = \frac{k_A}{k_B} \tag{2.6}$$

and that optimum separation occurs when $k_A k_B = 1$. If $k_A = 100$ and $k_B = 0.01$, then $\alpha = 10^4$ and $k_A k_B = 1$. This would indicate a good separation because 1% of A would remain unextracted and 1% of B would be extracted. However, if $k_A = 1.0$ and $k_B = 10^{-4}$, we still obtain an α of 10^4 but $k_A k_B = 10^{-4}$, meaning that 50% of A is in each phase and 0.01% of B is extracted. Solution B has been significantly unextracted but not separated from A. This second example of a separation lends itself to countercurrent distribution (extraction). Craig (17–20) can be credited with the refinement of this technique. This extraction technique can be used to partially explain what occurs in a chromatographic column. It also is illustrative for explaining zone broadening in multistage processes. What one assumes is that the system is made of individual, discontinuous steps (theoretical plates) and that the system comes to equilibrium as solute passes from one step (plate) to the next. Thus it is referred to as the "plate" model. This model and the "rate" model (discussed in Section 2.3.2) may both be used to describe the theory of chromatography. Both models arrive at the same basic conclusion that zone broadening is proportional to the square root of the column length and that the zone shape follows the normal distribution law. Figure 2.19 illustrates the similarity between the countercurrent extraction (CCE) process and the chromatographic process.

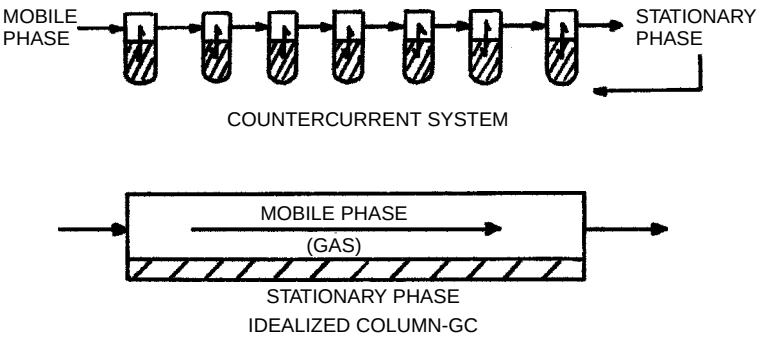

FIGURE 2.19 Comparison of countercurrent extraction and the chromatographic process.

The concept of plate theory was originally proposed for the performance of distillation columns (21). However, Martin and Synge (22) first applied the plate theory to partition chromatography. The theory assumes that the column is divided into a number of zones called *theoretical plates.* One determines the zone thickness or height equivalent to a theoretical plate (HETP) by assuming that there is perfect equilibrium between the gas and liquid phases within each plate. The resulting behavior of the plate column is calculated on the assumption that the distribution coefficient remains unaffected by the presence of other solutes and that the distribution isotherm is linear. The diffusion of solute in the mobile phase from one plate to another is also neglected.

Martin and Synge (22) derived an expression for the total quantity q_n of solute in plate n and the volume of mobile phase (carrier gas) that passes through the column:

$$q_n = \frac{1}{(2\pi N)^{1/2}} \exp\left\{\frac{-[(V/v_R) - N]^2}{2N}\right\} \tag{2.7}$$

where v_R is the retention volume per plate and V is the total retention volume.

If $q_n = v_R C_n$, where C_n is solute concentration in the mobile phase of plate n, then

$$C_n = \frac{1}{v_R(2\pi N)^{1/2}} \exp\left\{\frac{-[(V/v_R) - N]^2}{2N}\right.$$

or

$$C_n = \frac{1}{(v_R\sqrt{N})\sqrt{2\pi}} \exp\left[\frac{-(V - Nv_R)^2}{2(v_R\sqrt{N})^{1/2}}\right] \tag{2.8}$$

Equation 2.8 has the form of the normal error curve, and from the geometric properties of the curve we can show that

$$N = 16\left(\frac{V_R}{w_b}\right)^2 \tag{2.9}$$

where w_b is the base width of the peak. Equation 2.9 is a measure of the efficiency of a gas chromatographic column. Sometimes the number of plates is measured at the bandwidth at half-height w_h. From statistics

$$W = \left(\frac{2}{\ln 2}\right)^{1/2} w_h \tag{2.10}$$

Equation 2.9 may be expressed as Equation 2.11 in terms of w_h:

$$\begin{aligned} N &= 8 \ln 2 \left(\frac{V_R}{w_h}\right)^2 \\ &= 8(2.30 \log 2)\left(\frac{V_R}{w_h}\right)^2 \\ &= 5.54\left(\frac{V_R}{w_h}\right)^2 \end{aligned} \tag{2.11}$$

If the number of theoretical plates in a column is known, it is possible to calculate the maximum sample size (i.e., the volume of sample that will not cause more than 10% peak broadening) that can be injected:

$$V_{\max} = a\frac{V_{\mathrm{m}}^0 + K V_{\mathrm{L}}}{\sqrt{N}} \tag{2.12}$$

where $V_{\mathrm{m}}^0 = j V_{\mathrm{m}}$, and a is a constant. In terms of the column internal diameter d_{c}, we obtain

$$V_{\max} = a\left(\frac{\pi}{4}\right) d_{\mathrm{c}}^2 L + \frac{K V_{\mathrm{L}}}{\sqrt{N}} \tag{2.13}$$

Equation 2.13 states that the maximum sample size is inversely related to the number of theoretical plates. This equation can be written more precisely as

$$V_{\max} = a\frac{V_{\mathrm{R}}^0}{\sqrt{N}} \tag{2.14}$$

which relates sample size to corrected retention volume. The constant a is determined experimentally by the successive injection of smaller samples until no more improvement in resolution is seen.

As long as the sample occupies less than $0.5(N)^{1/2}$ theoretical plates, there will be no band broadening because of sample size. As the total number of theoretical plates increases within a column, the maximum space (in terms of theoretical plates) that should be occupied by the sample will also increase. However, the percentage of column length available for sample will decrease (because the number of theoretical plates per column length increases), as shown in Table 2.1.

Having calculated the number of theoretical plates and knowing the length of the column, one may determine the HETP:

$$H = \mathrm{HETP} = \frac{L}{N} = \frac{L}{16}\left(\frac{w_{\mathrm{b}}}{V_{\mathrm{R}}}\right)^2 = \frac{L}{16}\left(\frac{w_{\mathrm{b}}}{t_{\mathrm{R}}}\right)^2 \tag{2.15}$$

Plate theory disregards the kinetics of mass transfer; therefore, it reveals little about the factors influencing HETP values. Plate theory tells us that HETP

TABLE 2.1 Sample Space in Terms of Theoretical Plates in Column

	Maximum Space Available for Sample	
Number of Plates in Column	In Terms of Theoretical Plates[a]	In Terms of % Column Length[b]
4	1	25
100	5	5
400	10	2.5
10,000	50	0.5

[a] $0.5\sqrt{N}$.
[b] $(0.5\sqrt{N}/N)100$.

becomes smaller with increasing flowrate; however, experimental evidence show that a plot of HETP versus flowrate always goes through a minimum.

The "theoretical plate" defined in GC is not the same as that in distillation or other countercurrent mass transfer operations. In the latter, the number of theoretical plates represents the number of equilibrium stages on the equilibrium curve of a binary mixture that causes a given concentration change. In other words, HETP is the length of column producing a concentration change that corresponds to one equilibrium stage. In GC, the number of theoretical plates is a measure of peak broadening for a single component during the lifetime of the column. For a given column of constant length, therefore, the HETP represents the peak broadening as a function of retention time. In a gas chromatographic column, each component will yield different N and HETP values. Those solutes with high retention (high K values) will result in greater numbers of theoretical plates and thus lower HETP values. It is generally found that the necessary number of theoretical plates for packed gas chromatographic columns is 10 times greater than in distillation for a similar separation.

Fritz and Scott (23) derived simple statistical expressions for calculating the mean and variance of chromatographic peaks that are still on a column (called *position peaks*) and these same peaks as they emerge from the column (called *exit peaks*). The classical plate theory is derived by use of simple concepts from probability theory and statistics. In this treatment, each sample chemical substance molecule is examined separately, whereas its movement through the column is described as a stochastic process. Equations are given for both discrete- and continuous-flow models. They are derived by calculating the mean and variance of a chromatographic peak as a function of the capacity factor k.

Using this statistical approach, Fritz and Scott studied two classical models falling into the category of plate theory: the discrete-flow model and the continuous-flow model. According to peak theory, the chromatographic column is considered to be divided into "plates" or "disjoint segments." In their discussion, those authors refer to these "disjoint segments" as *theoretical segments* (TSs). Therefore, the sample molecules move from one TS to the next until they reach the last segment from which they elute from the column.

1. *Discrete-Flow Model.* This model requires several assumptions: (a) all the mobile phase moves from one segment to the next segment at the end of a discrete interval and (b) the sample molecules are always in equilibrium with the mobile and stationary phases. On the basis of these assumptions, the equilibrium condition expresses the probability p that the molecule is in the mobile phase

$$p = \frac{1}{1+k} \tag{2.16}$$

and the probability $(1 - p)$ that the molecule is in the stationary phase:

$$1 - p = \frac{k}{1+k} \tag{2.17}$$

2. *Continuous-Flow Model*. The assumptions in this model are that (a) the mobile and stationary phases remain in equilibrium throughout the separation, (b) the mobile phase flows from one segment to the next segment at a constant rate, and (c) perfect mixing takes place in all segments.

Theoretical plate number N and effective theoretical plate number N_{eff} may then be calculated for both the discrete- and continuous-flow models. A number of chromatographic systems from the literature were examined by Fritz and Scott. In all cases they demonstrated the applicability of the actual data to their system.

2.3.1.1 Discrete-Flow Model

$$N = \frac{[E(T)]^2}{\text{var}(T)} = \frac{[r(1+k)]^2}{rk(1+k)} = r\left(\frac{1+k}{k}\right) \tag{2.18}$$

$$N_{\text{eff}} = \frac{[E(T)-t_0]^2}{\text{var}(T)} = \frac{(rk)^2}{rk(1+k)} = r\left(\frac{k}{1+k}\right) \tag{2.19}$$

2.3.1.2 Continuous-Flow Model

$$N = \frac{[E(T)]^2}{\text{var}(T)} = \frac{[r(1+k)]^2}{r(1+k)^2} = r \tag{2.20}$$

$$N_{\text{eff}} = \frac{[E(T)-t_0]^2}{\text{var}(T)} = \frac{(rk)^2}{r(1+k)^2} = r\left(\frac{k}{1+k}\right)^2 \tag{2.21}$$

where $E(T)$ = expected exit time of sample substance
$\text{var}(T)$ = variance of time
r = number of theoretical segments in column
k = capacity factor
t_0 = exit time for nonsorbed substance

Therefore, only the plate numbers N for the continuous-flow model are independent of the capacity factor k (Equation 2.20).

2.3.2 Rate Theory

Although HETP is a useful concept, it is an empirical factor. Since plate theory does not explain the mechanism that determines these factors, we must use a more sophisticated approach, the rate theory, to explain chromatographic behavior. Rate theory is based on such parameters as rate of mass transfer between stationary and mobile phases, diffusion rate of solute along the column, carrier-gas flowrate, and the hydrodynamics of the mobile phase.

Glueckauf (24) studied the effect of four factors on the chromatographic process: (1) diffusion in the mobile phase normal to the direction of flow, (2) longitudinal diffusion in the mobile phase, (3) diffusion into the particle, and (4) size of the particle.

The interpretation of the resulting chromatogram will indicate how well a separation has been performed. This interpretation can be viewed from two points: (1) how well the centers of the solute zones have been disengaged and (2) how compact the resulting zones are. Many chromatographic separations accomplish the first point but not the second, which results in the two zones spreading into each other.

We consider the three variables that cause zone spreading: ordinary diffusion, eddy diffusion, and local nonequilibrium. We approach this discussion from the random-walk theory, since the progress of solute molecules through a column may be viewed as a random process.

First we define these three types of diffusion:

1. *Ordinary Diffusion.* This process results when there exists a region of high concentration and a region of low concentration. The migration is from the higher to the lower concentration region in the axial direction of the column. Diffusion occurs on the molecular level, resulting from movement of molecules after collision. Once the sample has been placed at the top of the column (in the minimum number of theoretical plates), these gradient regions exist.

2. *Eddy Diffusion.* Visualize a column packed with marbles of equal diameter. The void space along the column is essentially uniform (74% of column volume is occupied with the marbles and 26% is open or void volume). As the size of the marbles (particles) decreases, it becomes increasingly difficult to control uniformity in size and to prevent crushing or fractionation of the particle. This is especially true for column support materials that are easily fractionated if excessive vibrating or tapping is used in the packing procedure. With the particle size used in analytical gas chromatographic columns, 60/80 mesh (0.25–0.17 mm) for 0.25-in.-i.d. columns and 110/120 mesh (0.13–0.12 mm) for 0.125-in.-i.d. columns, it is very difficult to have all the particles of the same diameter, and some of these particles might fit into void spaces between particles. The overall effect is that the spaces along the column are not uniform. When a sample migrates down the column, therefore, each molecule "sees" different paths and each path is of a different length. Some molecules take the longer paths and others take the shorter paths. There are also variations in the velocities of the mobile phase within these pathways. The overall result is that some molecules lag behind the center of the zone, whereas others move ahead of the zone. Therefore, the eddy diffusion process results from flow along randomly spaced variable-size particles in the column.

3. *Local Nonequilibrium.* As the zone of solute molecules migrates through the column (approximating a Gaussian curve), there exists a variable concentration profile from the leading edge through the center to the trailing edge. As this zone continues to migrate down the column, it is constantly bringing an ever-changing concentration profile in contact with the next part of the column. This effect results in different rates of equilibration along the column. Thus each section (theoretical plate) in the column is constantly attempting to equilibrate with a variable concentration zone in the mobile phase. At one

time the zone attempts to equilibrate with a low concentration in the mobile phase, and then at another time with a high concentration. If no flow were present, equilibration would proceed; however, we are in a dynamic system and there is always flow. These overall processes result in nonequilibrium at each theoretical plate. The overall process is determined by kinetic rate processes that account for transfer of the solute molecules between the two phases in the column; that is, the mass transfer rate from mobile phase to stationary phase is different from the mass transfer rate from the stationary phase to the mobile phase.

Viewing the zone migration as discussed previously, we can conclude that increasing the mobile-phase velocity will increase the nonequilibrium effect, providing for more rapid exchange of solute molecules between the mobile and stationary phases and thus decreasing the nonequilibrium effect. Theory tells us that horizontal displacement (perpendicular to flow) is constant throughout the zone, proportional to velocity of flow, but inversely proportional to rate of restoring equilibrium. On the other hand, vertical displacement (parallel to flow) is proportional to the concentration gradient.

Since the three processes discussed earlier are all random diffusion processes, we can evaluate the zone broadening from the viewpoint of a random walk. If a process results from the random back–forth motion of solute molecules, we have a concentration profile that is Gaussian in shape (i.e., the number of molecules preceding the zone center equals the number of molecules trailing the zone center). The extent of spreading for normal Gaussian distributed molecules is described by the standard deviation σ. This bandspreading σ is defined in random-walk model by the number of steps taken n and the length of each step l:

$$\sigma = ln^{1/2} \tag{2.22}$$

This equation states that zone spreading is proportional to step length but not to the number of steps. For instance, movement is random; it takes 16 steps to give a displacement 4 times the average length of each step.

We know from statistical treatments that standard deviations are not additive. However, variances, the square of the standard deviation, are additive. In terms of the chromatographic process, three diffusive process variables contribute to zone spreading. Thus we can sum these variables in terms of variances to give the overall contribution of zone spreading. The combined effect may be shown as

$$\sigma^2 = \Sigma\sigma_i^2 \tag{2.23}$$

where the $\Sigma\sigma_i$ term is a sum of each of the three processes: σ_D for ordinary diffusion, σ_E for eddy diffusion, and σ_K for nonequilibrium diffusion effects.

The ordinary diffusion process term is defined by the Einstein diffusion equation:

$$\sigma_D^2 = 2Dt \tag{2.24}$$

where D is the coefficient of diffusion and t represents the time that molecules spend in the mobile phase from the start of the random process. The term t also can be expressed in terms of the distance that the zone has moved L and the velocity of the mobile phase u; thus

$$t = \frac{L}{u} \tag{2.25}$$

and Equation 2.24 becomes

$$\sigma_D^2 = \frac{2DL}{u} \tag{2.26}$$

The reader should keep in mind when developing a theory of zone spreading that we must have a point of reference to show how the spreading develops. This point of reference is the zone center.

The eddy diffusion term σ_E describes the change in pathway and velocity of solute molecules in reference to the zone center. If the molecules are in a "fast" channel, they can migrate ahead of the zone center; if in a "slow" channel, they can lag behind the zone center. To quantify the eddy diffusion term, we must describe the step length and the number of steps taken in a specified period of time. The void or channel volume between particles would be expected to be in the order of one particle diameter d_p. As molecules move from one channel to another, their velocity will be of the order of $+d_p$ *or* $-d_p$ (with respect to the zone center). So, on average, the molecules will take an equivalent step of d_p.

We can determine the number of steps in terms of the total column length L and the equivalent length of the step; therefore

$$n = \frac{L}{d_p} \tag{2.27}$$

On reflection it is apparent that channels cannot be regarded as either "fast" or "slow." Rather, there will be a range of velocities with some average value for the entire column length. Also, the column voids or channels will not be exactly equal to d_p, but will vary from larger than d_p to smaller than d_p, with an overall average of d_p. In light of the preceding description we can equate d_p for length of step l and L/d_p for number of steps. Substitution into Equation 2.22 gives

$$\sigma_E = d_p \left(\frac{L}{d_p}\right)^{1/2} = (Ld_p)^{1/2} \tag{2.28}$$

This equation states that eddy diffusional effects on zone spreading increase with the square root of zone displacement and particle size.

Equations 2.24 and 2.28 account for the effect of ordinary and eddy diffusion in the zone-broadening process. Now we need to express nonequilibrium effects that are concerned with the time that the solute molecules spend in the two

phases. Let us define a few more terms in order to set up some mathematical relationships:

k_1 = transition rate of the molecule from mobile phase to stationary phase
$1/k_1$ = average time required for one sorption to occur
k_2 = transition rate of molecules from stationary phase to mobile phase
$1/k_2$ = time required for one desorption to occur

A molecule in the mobile phase is moving faster than the center of the zone. The velocity of the zone is Ru, where R is the fraction of solute molecules in mobile phase and u is the mobile-phase velocity. Therefore, $1 - R$ is the fraction of solute molecules in the stationary phase with a velocity of zero. Now, molecules move back and forth with respect to the zone center as each phase transfer occurs. In terms of random walk, n is the number of transfers our molecules take between the two phases. In terms of sorptions–desorptions, n is twice the number of desorptions (one desorption occurs for each sorption), and the time needed for the solute zone to move through the column (distance $= L$) at its velocity Ru is

$$t = \frac{L}{Ru} \tag{2.29}$$

During this time t, the molecules will spend the fraction R in mobile phase and the fraction $(1 - R)$ in the stationary phase. So the time that the fraction of molecules $(1 - R)$ spend in the stationary phase will be

$$t = \frac{(1 - R)L}{Ru} \tag{2.30}$$

The number of desorptions is the time spent by the molecules in the stationary phase (Equation 2.30) divided by $1/k_2$:

$$n_{\text{des}} = \frac{(1 - R)L/Ru}{1/k_2} = \frac{k_2(1 - R)L}{Ru} \tag{2.31}$$

Since there are twice as many phase transfers as there are desorption processes, the number of steps n is equal to two times Equation 2.31, or

$$n = \frac{2k_2(1 - R)L}{Ru} \tag{2.32}$$

To obtain a value for the distance a molecule moves back with respect to the zone center l, we need to consider $1/k_2$, the lifetime of a molecule in the stationary phase. The center of the zone moves forward $[Ru \times (1/k_2)]$ or (Ru/k_2) during the time that the molecule is in the stationary phase; thus our steplength also is Ru/k_2. By similar reasoning we arrive at the same value for the forward movement of molecules ahead of the zone center.

We now can describe an equation for the effect of nonequilibrium on zone spreading viewed as a random walk. Substituting Ru/k_2 for l and $2k_2(1- R)L/Ru$

for n in Equation 2.22, we have

$$\sigma_k = \frac{Ru}{k_2}\left[\frac{2k_2(1-R)L}{Ru}\right]^{1/2}$$
$$= \left[\frac{2R(1-R)Lu}{k_2}\right]^{1/2} \tag{2.33}$$

Equation 2.33 indicates that an increase in flow velocity causes an increase in nonequilibrium effects. Provision for rapid exchange of solute molecules between phases decreases these effects.

We may now return to Equation 2.23 and make the appropriate substitutions from Equations 2.24, 2.28, and 2.33:

$$\sigma^2 = 2Dt + Ld_p + \frac{2R(1-R)Lu}{k_2} \tag{2.34}$$

$$t = \frac{L}{u} \qquad \text{(Equation 2.25);} \quad \therefore$$

$$\sigma^2 = \frac{2DL}{u} + Ld_p + \frac{2R(1-R)Lu}{k_2} \tag{2.35}$$

$$\sigma^2 = L\left[\frac{2D}{u} + d_p + \frac{2R(1-R)u}{k_2}\right] \tag{2.36}$$

Martin and Synge (2) introduced height equivalent to a theoretical plate H as a measure of zone spreading:

$$H = \frac{\sigma^2}{L} \tag{2.37}$$

So Equation 2.36 may be written as

$$H = \frac{2D}{u} + d_p + \frac{2R(1-R)u}{k_2} \tag{2.38}$$

Rearrangement of Equation 2.38 yields

$$H = d_p + \frac{2D}{u} + \frac{2R(1-R)u}{k_2} \tag{2.39}$$

To find the correct flow velocity u, which gives the minimum plate height H_{min}, we take the first derivative of Equation 2.38 and set dH/du equal to zero. This results in

$$u = \left[\frac{k_2 D}{R(1-R)}\right]^{1/2} \tag{2.40}$$

The van Deemter equation (25) is used for describing the gas chromatographic process. This equation was evolved from the earlier work (26) and was later

extended with Glueckauf's theory. The equation was derived from consideration of the resistance to mass transfer between the two phases as arising from diffusion:

$$H = \frac{2D_c}{u} + \left(\frac{8}{\pi^2}\right)\frac{k}{(1+k)^2}\left(\frac{d_f^2}{D_l}\right)u \tag{2.41}$$

where D_c = overall longitudinal diffusivity of solute in gas phase
k = capacity factor
d_f = effective film thickness of liquid phase
D_l = diffusivity of solute in liquid phase
u = apparent linear flowrate of gas phase

The first term in Equation 2.41 is the contribution due to overall longitudinal diffusion, and the second term is contribution due to resistance to mass transfer in the liquid phase.

The overall longitudinal diffusivity D_c is the sum of apparent longitudinal diffusivity D_a and true molecular diffusivity D_g:

$$D_c = D_a + \gamma D_g \tag{2.42}$$

The factor γ is used to account for irregular diffusion patterns and usually is less than unity because molecular diffusivity is smaller in packed columns than in open tubes.

Klinkenberg and Sjenitzer (27) showed statistically that

$$D_a = \lambda u d_p \tag{2.43}$$

where λ is a dimensionless constant characteristic of packing. This equation is indicative of how poor or effective the packing homogeneity is in the column; for regular packings, $\lambda < 1$; for nonuniform packed columns with channels, $\lambda > 1$. The term d_p is the particle diameter in centimeters.

Uneven distribution of the stationary phase liquid on the solid support particles causes band dispersion. This may be rationalized if one considers that molecules entering a thin part of the liquid film permeate faster than in a thicker part of the liquid film. This effect causes some molecules to spend more time in the liquid phase than other molecules. This slow movement through the column results in spreading of the band.

Considering all the effects discussed above and combining Equations 2.41 and 2.43, we have the expression

$$H = 2\lambda d_p + \frac{2\gamma D_g}{u} + \frac{8}{\pi^2}\frac{k}{(1+k)^2}\left(\frac{d_f^2}{D_l}\right)u \tag{2.44}$$

This equation predicts that for maximum column performance, we must minimize the contribution of each term while maintaining a constant linear flowrate.

The first term accounts for the geometry of the packing, the second for longitudinal diffusion in the gas phase, and the third for resistance to mass-transfer processes.

The general form for the van Deemter equation is

$$H = A + \frac{B}{u} + Cu \tag{2.45}$$

where $A = 2\lambda d_p$ = eddy diffusion term
$B = 2\gamma D_g$ = longitudinal or ordinary diffusion term
$C = (8/\pi^2)[k/(1+k)^2](d_f^2/D_l)$
= nonequilibrium or resistance to mass transfer term

A representation of this equation is given in Figure 2.20, which shows the effect of H with changes in linear gas velocity. Equation 2.45 represents a hyperbola that has a minimum at velocity $u = (B/C)^{1/2}$ and a minimum H value (H_{min}) at $A + 2(BC)^{1/2}$. The constants may be graphically calculated from an experimental plot of H versus linear gas velocity as shown in Figure 2.20.

It is also useful to plot H against $1/u$ (Figure 2.21). In both presentations (Figures 2.20, 2.21) the intercept of the linear portion of the H plot will equal $2\lambda d_p$. Thus, if the particle size d_p is known, λ can be calculated and a measure of packing regularity obtained. From the slope of the linear part of the $H-u$ curve one also can estimate the film thickness d_f, if D_l and k are known (resistance to mass transfer in liquid-phase term).

The constants A, B, C can also be determined by the method of least squares. A gradual approximation of B may be calculated from a plot of $H-Cu$ versus $1/u$, *and* C can be approximated from a plot of $H-Bu$ versus u.

Let us take a better look at the effect of the terms of Equation 2.44 on plate height. The contribution of the $2\lambda d_p$ term can be decreased by reducing the particle size. As the particle size becomes smaller, however, the pressure drop through

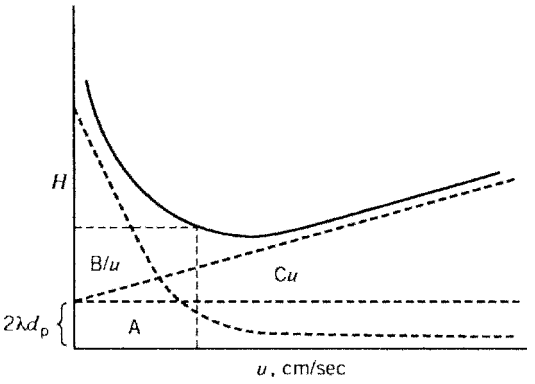

FIGURE 2.20 Van Deemter plot. Change in H versus linear gas velocity u: $H_{min} = A + (2BC)^{1/2}$; $u_{opt} = (BC)^{1/2}$.

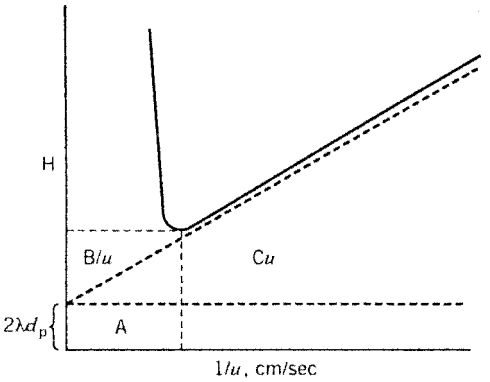

FIGURE 2.21 Rate theory equation plotted as H versus $1/u$: $H_{\min} = A + (2BC)^{1/2}$; $u_{\text{opt}} = (BC)^{1/2}$.

the column increases. The value of λ usually increases as d_p decreases. Of the three terms in Equation 2.44, only this first one is independent of linear flowrate.

The second term, $2\gamma D_g/u$, is a measure of the effect of molecular diffusion on zone spreading. This term may be decreased by reducing the molecular diffusivity D_g. We know from the kinetic theory of gases that the D_g value depends on the nature of the vapor and the temperature and the pressure of the system. Diffusion in low-molecular-weight gases (H_2 and He) is high compared to that in higher-molecular-weight gases (N_2 or CO_2). If this were the only criterion for choice of carrier gas, one would choose N_2 or CO_2 rather than He. This is evidenced by the fact that optimum gas velocity is governed by $(B/C)^{1/2}$. One obtains a value for $(B/C)^{1/2}$ by differentiating Equation 2.45 with respect to u and then setting $dH/du = 0$; $u_{\text{opt}}(H_{\min})$ is then equal to $(B/C)^{1/2}$. However, other factors affect the choice of a carrier gas, such as the effect of the sensitivity of the detector employed. If in a particular system the C term is small and high flowrates are allowable (thus reducing term $2\gamma D_g/u$), the nature of the carrier is not too important. For columns of low permeability, a low-molecular-weight (less viscous) gas might be the best choice (e.g., He). If the C term is large and low flowrates are used, the term $2\gamma D_g/u$ becomes important and the carrier gas can exert influence on the HETP. In this case, high-molecular-weight gases (e.g., N_2 or CO_2) would be preferred because solute diffusion coefficients would be small.

The third term of Equation 2.44 accounts for resistance to mass transfer in the liquid phase. An obvious way of reducing this term is to reduce the liquid film thickness d_f. This causes a reduction in k and an increase in the term $k/(1+k)^2$. However, the use of thinly coated column packings increases the probability of adsorption of solute molecules on the surface of support material, which might result in tailing of peaks.

The k term is temperature dependent, so we increase k and decrease $k(1+k)^2$ by lowering of temperature. Lowering the temperature increases viscosity and thus decreases D_l. Therefore, the effects of the factors $k/(1+k)^2$ and $1/D_l$ counteract each other.

Thus it can be seen that the observed HETP not only is a function of column packing but also depends on operating conditions and the properties of the solute. This is why different values of HETP (or different numbers of theoretical plates per unit column length) are obtained for various solutes.

Many modifications to the original van Deemter plate height equation have appeared in the literature (28–32). Some account for mass transfer in the gas phase (28,29), and other modifications have been made for velocity distribution because of flow retardation of interfacial resistance (30,31). Improvements were attempted, usually stochastic theories based on random-walk theory (32). However, we elaborated on the work of Giddings (33), who described plate height contributions as a function of the diffusional character of zone broadening by accounting for local nonequilibrium.

2.3.2.1 Modifications of the van Deemter Equation

If one accounts for the fact that resistance to mass transfer can occur in the stationary phase as well as in the mobile phase, Equation 2.45 may be written as

$$H = A + \frac{B}{u} + C_l u + C_g u \tag{2.46}$$

The last term accounts for the resistance to mass transfer in the gas phase. Low-loaded liquid coatings cause the C_g term to be significant. Equation 2.46 was further extended to account for velocity distributions due to gas flow retardation in the layers C_1 and the interaction of the two types of gas resistance C_2:

$$H = A + \frac{B}{u} + C_l u + C_g u + C_1 u + C_2 u \tag{2.47}$$

The term $C_g u$ may be defined as

$$C_g u = c_a \frac{k^2}{(1+k)^2} \left(\frac{d_g^2}{D_g} \right) u \tag{2.48}$$

where c_a is a proportionality constant, d_g is the gas diffusional pathlength, and D_g is the diffusion coefficient of solute molecules in the gas phase.

The C_1 term becomes significant with rapidly eluted but poorly sorbed components. The value of C_1 depends on the particle size of the packing

$$C_1 u = \left(\frac{c_b d_p^2}{D_g} \right) u \tag{2.49}$$

where c_b is a proportionality factor approximately equal to unity. Giddings and Robinson (34) realized that the processes in the gas phase cannot be considered independent with respect to their effect on H. Thus they stated that the term A (flow characteristic) and the effect of resistance to mass transfer in the gas phase must be treated dependently. So Equation 2.46 becomes

$$H = \frac{1}{1/A + 1/C_1 u} + \frac{B}{u} + C_l u + C_g u + H_e \tag{2.50}$$

The term

$$\frac{1}{1/A + 1/C_1 u}$$

results from the merging of the eddy diffusion term and the velocity distribution term ($C_1 u$) of Equations 2.47 and 2.49. The term H_e is introduced to account for the characteristics of the equipment used in the system. The first term (Equation 2.50) is not simple (35,36). Depending on the nature of the packing and the flow, five possible mechanisms can take place, so our term becomes a summation term:

$$H = \sum_{i=1}^{5} \frac{1}{(1/A) + 1/C_1 u} + \frac{B}{u} + C_l u + C_g u + H_e \tag{2.51}$$

The five possible mechanisms of band broadening occur because of flow (1) through channels between particles, (2) through particles, (3) resulting from uneven flow channels, (4) between inhomogeneous regions, and (5) throughout the entire column length.

All preceding discussion has assumed no compressibility of the gas stream. With columns where the pressure drop is large, the change in gas velocity should be considered. (Gas expansion also causes zone spreading.) DeFord et al. (37) demonstrated the importance of a pressure correction and after considering the A term to be negligible, developed the following equation:

$$H = \frac{B^0}{p_0 u_0} + (C_g^0 + C_1^0) p_0 u_0 f + C_l^0 u_0 j \tag{2.52}$$

where B^0, C_g^0, C_1^0, C_l^0 = coefficients determined by measuring H for various outlet pressures and outlet velocities

p^0 = outlet pressure

u_0 = outlet gas velocity

j = James–Martin pressure correction factor (compressibility factor)

$$= \frac{3}{2}\left[\frac{(p_i/p_0)^2 - 1}{(p_i/p_0)^3 - 1}\right]$$

f = pressure correction

$$= p_i(p_0 + 1)j^2/2$$

f = usually unity and can be neglected except in accurate theoretical work

2.3.2.2 Flow

The rate at which zones migrate down the column is dependent on equilibrium conditions and mobile-phase velocity; on the other hand, how the zone broadens depends on flow conditions in the column, longitudinal diffusion, and the rate of mass transfer. Since various types of columns are used in GC—namely, open

tubular columns, support-coated open tubular columns, packed capillary columns, and analytical packed columns—we should study the conditions of flow in a gas chromatographic column. Our discussion of flow is restricted to Newtonian fluids, that is, those in which the viscosity remains constant at a given temperature.

Flow through an open tube is characterized by the dimensionless Reynolds number

$$\mathrm{Re} = \frac{\rho d u}{\eta} = \frac{dG}{\mu} \tag{2.53}$$

where ρ = fluid density (g/mL)
d = tube diameter (cm)
u = fluid velocity (cm/s)
η = fluid viscosity (poise)
G = mass velocity (g/cm$^2 \cdot$ s)
μ = absolute viscosity (g/cm $\cdot$ s)

Inertial forces of the fluid increase with density and the square of velocity (ρu^2), whereas viscous forces decreases with increasing diameter of tube ($\eta u/d$) and increase with viscosity and velocity. High Reynolds numbers (Re $>$ 4000) result in turbulent flow; with low Reynolds number (Re $>$ 2000), the flow is laminar. Laminar flow results from formation of layers of fluid with different velocities after a certain flow distance, as illustrated in Figure 2.22, segment A. Flow at the walls is zero and increases on approach to the center of the tubes. The laminar flow pattern results from mobile-phase layers with different velocities traveling parallel to each other. The maximum flow at the center is twice the average flow velocity of fluid. Molecules in the field can exchange between fluid layers by molecular diffusion. Most open tubular columns operate under laminar flow conditions.

Turbulent flow results because of the radical mixing of layers to equalize flowrates. The mixing of the layers is due to the increased eddies, and mass transfer occurs by eddy diffusivity. Turbulent diffusivity increases in proportion to mean flow velocity, as depicted in Figure 2.22, segment B. Figure 2.22, segment C represents plug flow, which is unattainable in practice but does suggest a model from which other flows may be considered. The flow usually attained in packed columns is illustrated in Figure 2.22, segment D.

A considerable difference exists between flow through an open column and a packed column, as illustrated in Figure 2.23. Darcy's law, which governs flow through packed columns, states that flow velocity is proportional to the pressure gradient:

$$u_0 = \frac{B^2}{\eta} \frac{p_\mathrm{i} - p_\mathrm{o}}{L} \tag{2.54}$$

True average fluid velocity may be expressed as

$$\overline{u} = \frac{B_0}{\epsilon \eta} \frac{p_\mathrm{i} - p_\mathrm{o}}{L} \tag{2.55}$$

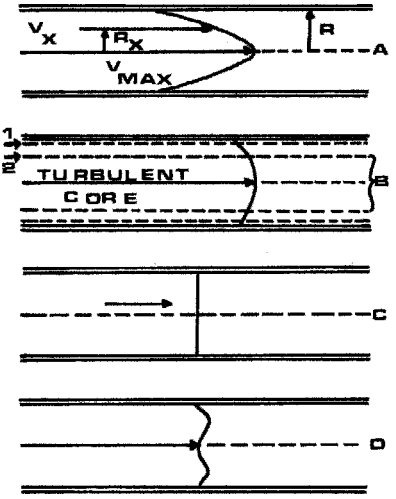

FIGURE 2.22 Flow profiles in tubes and packed columns. Segment A, laminar flow: r = tube radius, V_x = stream path velocity at radial position r_x, V_{max} = maximum flow velocity at tube center. Most open tubular columns operate with this profile. Segment B, turbulent flow: 1 = laminar sublayer, 2 = buffer layer. Segment C, plug flow. Segment D, flow in a packed column. Effect is more pronounced with smaller tube diameter:–particle size ratios.

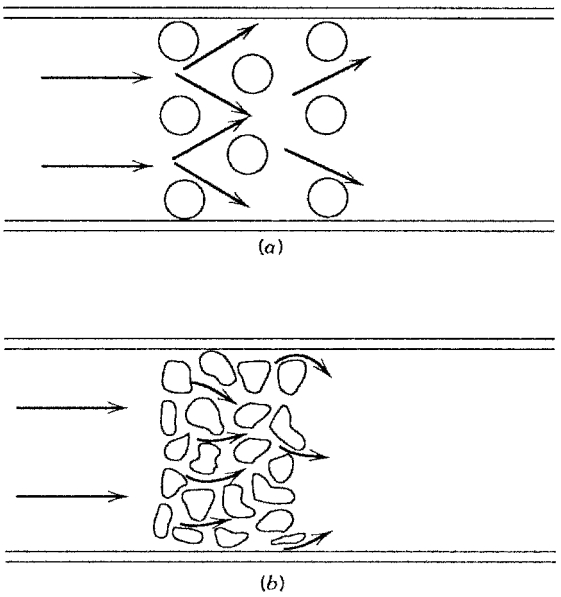

FIGURE 2.23 Representative flow through a packed column. (a) Simplified diagram of column with "uniform" particles. (b) Representative diagram of column with experimental particles. The degree of tortuosity of the path becomes dependent on the particle packing structure. Plug flow usually results.

and mean velocity of a fluid is represented by

$$\overline{u} \text{ (mean velocity)} = \frac{(p_i - p_o)r^2}{8\eta L} \tag{2.56}$$

Combination of Equations 2.54 and 2.56 gives the specific permeability coefficient B^0:

$$B_0 = \frac{r^2}{8} \tag{2.57}$$

If we express the free cross section of the column bed by the interparticle porosity (knowing that the total porosity of packed beds with porous particles is larger because of intraparticle space), we can obtain the true average fluid velocity overbar u:

$$\overline{u} = \frac{B_0(p_i - p_o)}{\epsilon, \eta L} \tag{2.58}$$

where $\overline{u}$ = superficial velocity (average velocity without packing)
B_2^0 = specific permeability coefficient (1 darcy = 10^{-8} cm^2)
ϵ = interparticle porosity (0.4 ± 0.03)
η = mobile-phase viscosity
L = column length
p_o = outlet pressure
p_i = inlet pressure

By combining the cross-sectional area of the tube $r^2\pi$ and the mean velocity (Equation 2.56), therefore, we can come up with an expression for the volumetric flowrate F_c:

$$F_c = \frac{(p_i - p_o)r^4\pi}{8\eta L} \tag{2.59}$$

Flow in packed columns may be expressed in terms of modified Reynolds numbers (Re)m, which take into account a geometric factor for the diameter of the particle rather than the diameter of the column (see Equation 2.53):

$$\text{Re}(m) = \frac{\rho u\, dp}{\eta} = \frac{dp\, G}{\mu} \tag{2.60}$$

For laminar flow, (Re)m values are less than 10 and with turbulent flow, (Re)$m > 200$. Packed gas chromatographic columns normally operate with a (Re)m of >10, so they may be considered to operate with laminar flow.

In gas chromatographic procedures the carrier-gas flow usually is measured after the column by a soap-bubble flowmeter. To obtain the average flowrate $\overline{F}_c$ in the column, one must account for three factors:

1. Compressibility correction j
2. Correction for flow being measured at room temperature T_0 rather than column temperature T, that is, T/T_0

3. Correction factor for vapor pressure of water p_w when using flowmeter $(p_0 - p_w)/p_0$, *where* p_0 is atmospheric pressure.

The initial flow F_0 into the column in terms of the measured flow F_c *is*

$$F_0 = F_c \left(\frac{T}{T_0}\right) \frac{p_0 - p_w}{p_0} \tag{2.61}$$

The average flowrate in the column is then determined by

$$\overline{F}_c = j F_c \left(\frac{T}{T_0}\right) \frac{p_o - p_w}{p_o} \tag{2.62}$$

This average flowrate term should be used to measure precise retention volumes.

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CHAPTER THREE

Columns: Packed and Capillary; Column Selection in Gas Chromatography

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PART 1 OVERVIEW

3.1 CENTRAL ROLE PLAYED BY COLUMN

The gas chromatographic column can be considered to be the central item in a gas chromatograph. Since the early 1970s the nature and design of the column has changed considerably from one containing either a solid adsorbent or a liquid deposited on an inert solid support packed into a length of tubing to one containing an immobilized or crosslinked stationary phase bound to the inner surface of a much longer length of fused-silica tubing. With respect to packing materials, solid adsorbents such as silica gel and alumina have been replaced by porous polymeric adsorbents while the vast array of stationary liquid phases in the 1960s was greatly reduced in number by the next decade to a smaller number of phases of greater thermal stability. These became the precursors of the chemically bonded or crosslinked phases of today. Column tubing fabricated from

copper, aluminum, glass, and stainless steel served the early analytical needs of gas chromatographers. Presently, fused-silica capillary columns having a length of 10–60 m and an inner diameter of 0.20–0.53 mm are in widespread use.

Although gas chromatography (GC) may be viewed in general as a mature analytical technique, improvements in column technology, injection, and detector design steadily appear nonetheless. Innovations and advancements in gas chromatography since the mid-1980s have been made with the merits of the fused-silica column as the focal point and have been driven primarily by the environmental, petrochemical, and toxicological fields as well as by advances in sample preparations and in mass spectrometry.

3.2 JUSTIFICATION FOR COLUMN SELECTION AND CARE

The cost of a gas chromatograph can range from $6000 to over $100,000 depending on the type and number of detectors, injection systems, and peripheral devices such as data system, headspace and thermal desorption units, pyrolyzers, and autosamplers. When one also factors in the purchase of high-purity gases on a regular basis required for operation of the chromatograph, it quickly becomes apparent that a sizable investment has been made in capital equipment. For example, cost-effectiveness and good chromatographic practice dictate that users of capillary columns should give careful consideration to column selection. The dimensions and type of capillary column should be chosen with the injection system and detectors in mind, considerations that are virtually nonissues with packed columns. Careful attention should also be paid to properly implemented connections of the column to the injector and detector and the presence of high boilers, particulate matter in samples, and other factors.

The price of a column ($200–$800) may be viewed as relatively small compared to the initial, the routine, and preventive-maintenance costs of the instrument. In fact, a laboratory may find that the cost of a set of air and hydrogen gas cylinders of research grade purity for FID operation is far greater than the price of a single conventional capillary column! Consequently, the column should be carefully selected for an application, handled with care following the suggestions of its manufacturer, and installed as recommended in the user's instrument manual to derive maximum performance from a gas chromatographic system.

The introduction of inert-fused silica capillary columns in 1979 markedly changed the practice of gas chromatography, enabling high-resolution separations to be performed in most laboratories. Previously such separations were achieved with reactive stainless-steel columns and with the glass columns. After 1979 the use of packed columns began to decline. A further decrease in usage of packed columns occurred in 1983 with the arrival of the megabore capillary column of 0.53 mm inner diameter, which serves as a direct replacement for the packed column. These developments, in conjunction with the emergence of immobilized or crosslinked stationary phases specifically tailored for fused silica capillary columns and overall improvements in column technology, have been responsible for the greater acceptance for capillary GC.

Trends The results of a survey of 12 leading experts in gas chromatography appeared in 1989 and outlined their thoughts on projected trends in gas chromatographic column technology, including the future of packed columns versus capillary columns (1). Some responses of that panel are

1. Packed columns are used for approximately 20% of gas chromatographic analyses.
2. Packed columns are employed primarily for preparative applications, fixed gas analysis, simple separations, and those separations where high resolution is not required or not always desirable (PCBs).
3. Packed columns will continue to be used for gas chromatographic methods that were validated on packed columns where time and cost of revalidation on capillary columns would be prohibitive.
4. Capillary columns will not replace the packed column in the near future, although few applications require packed columns.

Majors, shortly thereafter in 1990, summarized the results of a more detailed survey on column usage in gas chromatography, this one, however, soliciting response from *LC*GC* readership (2). Some conclusions drawn from this survey include

1. Nearly 80% of the respondents use capillary columns.
2. Capillary columns of 0.25 and 0.53 mm i.d. are the most popular as are columns lengths of 10–30 m.
3. The methyl silicones and poly(ethylene glycol) stationary phases are the most preferred for capillary separations.
4. Packed columns are used mostly for gas–solid chromatographic separations such as gas analyses.
5. The majority of respondents indicated the need for stationary phases of higher thermal stability.

Although no additional surveys have been published since 1990, the above mentioned trends still prevail to date for several reasons: (1) description of packed columns and related supplies and accessories have substantial presence in catalogs and Websites of the major column vendors and (2) the usage of packed columns users is abundant and has become apparent to the editors of this text after discussions with attendees in their GC short course offered at professional meetings.

Column manufacturers rely on the current literature, results of their own marketing surveys, the number of clicks on their Websites and so forth to keep abreast of the needs of practicing gas chromatographers. The fused silica capillary column has clearly emerged as the column of choice for most gas chromatographic applications. A market research report (3) showed that $100 million were spent on capillary columns worldwide and, at an estimated average cost of $400 for a column, this figure represents about 250,000 columns, just in 1993 alone. The

number of columns and users has considerably increased since then. Despite the maturity of capillary GC, instrument manufacturers continue to improve performance of gas chromatographs, which has diversely extended the applications of gas chromatography.

Chromatographers can expect to see continued splendid efforts by capillary column manufacturers on producing columns having lower residual activity and being capable of withstanding higher column temperature operation with reduced column bleeding. With the increasing popularity of high-speed or fast GC (Chapter 5) and increasing presence of GCMS (Chapter 7) in the analytical laboratory, especially for environmental, food, flavor, and toxicological analyses, improvements in column performance that affect the MS detector have steadily evolved, namely, columns with reduced column bleed. There is also an increased availability of capillary columns exhibiting stationary phase selectivity tuned for specific applications obtained by synthesis of new phases (4), the blending of stationary phases, and preparation of phases with guidance from computer modeling (5).

3.3 LITERATURE ON GAS CHROMATOGRAPHIC COLUMNS

The primary journals where developments in column technology and applications are published in hardcopy format and online versions include *Analytical Chemistry*, *Journal of Chromatography A*, *Journal of Chromatographic Science*, *Journal of Separation Science* (formerly the *Journal of High Resolution Chromatography*, including the *Journal of Microcolumn Separations*) and *LC*GC* magazine. The biennial review issue of *Analytical Chemistry*—Fundamental Reviews (published in even-numbered years) contains concise summaries of developments in gas chromatography. An abundance of gas chromatographic applications may be found in the companion issue, Application Reviews (published in odd-numbered years) covering the areas of polymers, geologic materials, petroleum and coal, coatings, pesticides, forensic science, clinical chemistry, environmental analysis, air pollution and water analysis.

Most industrial and corporate laboratories as well as college and universities have access to literature searching through one of a number of online computerized database service, such as Sci Finder Scholar. Although the location of articles on gas chromatography in primary Journals is relatively easy, finding publications of interest in lesser known periodicals can be a challenge and prove to be tedious at times. *CA Selects* and *Current Contents* are convenient alternatives. The biweekly *CA Selects*—Gas Chromatography topical edition available from Chemical Abstracts Service, is a condensation of information reported throughout the world. *Current Contents* in media storage formats provides weekly coverage of current research in the life sciences, clinical medicine, the physical, chemical, and earth sciences as well as agricultural, biology, and environmental sciences.

The periodic commercial literature and annual catalog of column manufacturers describing applications for their columns also contains more and more useful

technical information of a generic nature with each passing year. In addition, this author strongly recommends *LC*GC Magazine* as a valuable resource in which not only timely technical articles appear but also sections devoted to "Column Watch" and troubleshooting for GC. However, the Internet has emerged as most extensive source of chromatographic information, particularly the Websites of the column manufacturers as described in the next section.

3.4 GAS CHROMATOGRAPHIC RESOURCES ON THE INTERNET

The World Wide Web (WWW) has provided us with copious amounts of information via retrieval with search engines offered by an Internet service provider (ISP) (6); The Net has impacted our everyday activities with convenience of communication by e-mail, online placement of orders for all types of items and many other tasks. There are numerous Websites on gas chromatography in general, gas chromatographic columns, gas chromatographic detectors, and so on; all one has to do is locate them via "surfing the Net." All manufacturers of gas chromatographic instrumentation, columns, and chromatographic accessories and supplies maintain and update their Website, which is also clearly indicated on their commercial literature. This author strongly suggests that you identify and regularly visit the Internet addresses of column manufacturers, for example, and "bookmark" the corresponding Websites. Internet addresses may change slightly from time to time or perhaps dramatically as the nature of any business changes today via expansion or consolidation. For example, there has been some consolidation in the GC column industry where J&W was purchased by Agilent Technologies, Chrompack by Varian, and Supelco by Sigma-Aldrich. Therefore, prudence suggests that it is impractical to list here the exact Web addresses of vendors because they probably will ultimately change with time. However, "homepages" are easily searchable and continually updated, serving as an outstanding source of reference material for the practicing chromatographer. Convenient listing of Websites and addresses of vendors may be found in the annual Buyers' Guide in *American Laboratory*.

A listing of GC resources available on the Internet is simply too overwhelming a task. Nonetheless, a sampling of resourceful guides and information which one can find on gas chromatographic sites includes

Free downloads of software: retention time locking, method translation
Technical libraries of chromatograms searchable by solute or class of solutes
Column cross-reference charts
Application Notes
Guides to column and stationary phase selection
Guides to column installation
Guides to derivatization
Troubleshooting guides
Guides for syringe, septa, ferrule, and inlet liner selection

Guides for setting up a gas chromatograph
Past presentations at professional meetings such as Pittcon

Of the plethra of informative, significant .com and .org. sites, one site deserves special mention because it serves as a path both for immediate assistance for an analyst and also for the continuing education of users of GC and HPLC, namely, *the Chromatography Forum* maintained by LC Resources (www.lcresources.com.) There are several message boards, including a GC message board (and a liquid chromatography message board along with several others) where one can post anonymously a chromatographic problem or question while another individual(s) can post a response, initiating a dialog of communications on the topic. This site offers broadening of one's knowledge of the technique, even for the experienced user and is a particularly valuable asset for an analyst working in an environment where an individual is the sole chromatography user or does not have access to other resources or assistance with technical problems.

PART 2 PACKED-COLUMN GAS CHROMATOGRAPHY

Packed columns are still utilized for a variety of applications in gas chromatography. A packed column consists of three basic components: tubing in which packing material is placed, packing retainers (such as glass wool plugs) inserted into the ends of the tubing to keep the packing in place and thirdly, the packing material itself. In Part 2 the role and properties of solid support materials, adsorbents, commonly used stationary phases and procedures for the preparation of packed columns are described. Factors affecting packed column performance are also presented.

3.5 SOLID SUPPORTS AND ADSORBENTS

3.5.1 Supports for GLC: Diatomaceous Types, Halocarbons

The purpose and role of the solid support is the accommodation of a uniform deposition of stationary phase on the surface of the support. The most commonly used support materials are primarily diatomite supports and graphitized carbon (which is also an adsorbent for GSC), to a lesser extent, Teflon, inorganic salts and glass beads. There is no perfect support material because each has limitations. Pertinent physical properties of a support for packed-column GC are particle size, porosity, surface area, and packing density. Particle size impacts column efficiency via the A term or eddy diffusion contribution in the van Deemter expression (Equation 2.44). The surface area of a support is governed by its porosity, the more porous supports requiring greater amounts of stationary phase

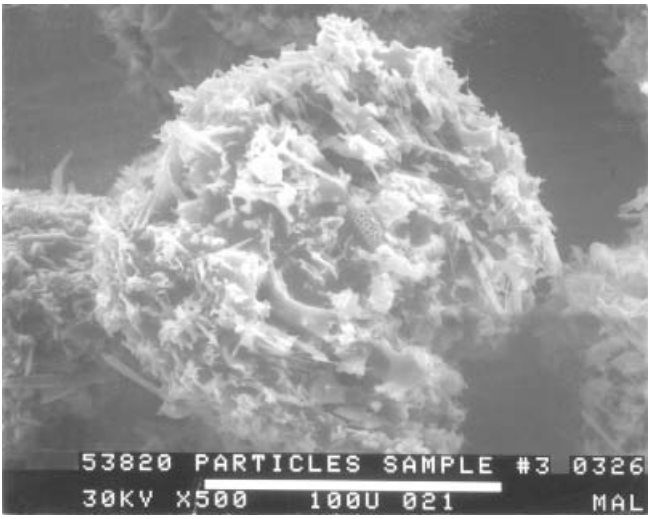


FIGURE 3.1 Scanning electron micrograph of 80/100-mesh Chromosorb W. (Reference 7.)

for surface coverage. A photomicrograph of Chromosorb W HP of 80/100 mesh appears in Figure 3.1, where the complex pore network is clearly evident.

3.5.1.1 Diatomite Supports

Basically, two types of support are made from diatomite. One is pink and derived from firebrick, and the other is white and derived from filter aid. German diatomite firebrick is known as *Sterchmal*. Diatomite itself is a diatomaceous earth, as is the German kieselguhr. Diatomite is composed of diatom skeletons or single-celled algae that have accumulated in very large beds in numerous parts of the world. The skeletons consist of a hydrated microamorphorous silica with some minor impurities (e.g., metallic oxides). The various species of diatoms number well over 10,000 from both freshwater and saltwater sources. Many levels of pore structure in the diatom cell wall cause these diatomites to have large surface areas (20 m^2/g). The basic chemical differences between the pink and white diatomite may be summarized as follows:

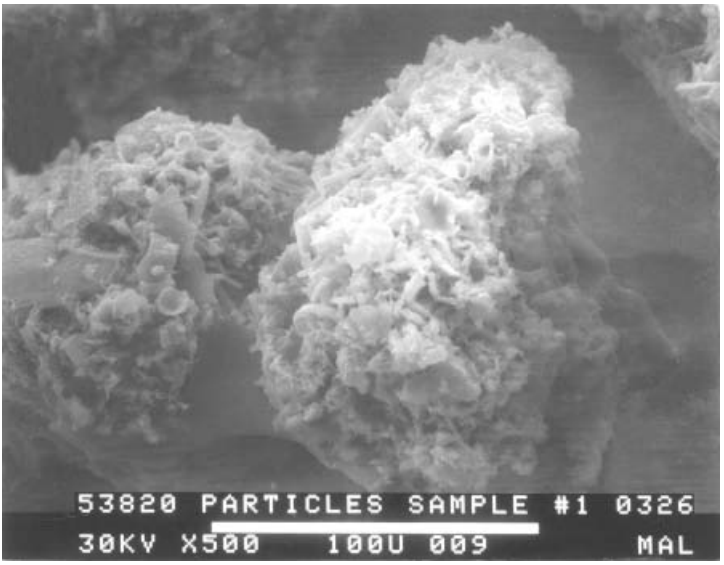
1. The white diatomite or filter aid is prepared by mixing it with a small amount of flux (e.g., sodium carbonate), and calcining (burning) at temperatures greater than 900°C. This process converts the original light gray diatomite to white diatomite. The change in color is believed to be the result of converting the iron oxide to a colorless sodium iron silicate.
2. The pink or brick diatomite has been crushed, blended, and pressed into bricks, which are calcined (burned) at temperatures greater than 900°C. During the process the mineral impurities form complex oxides and silicates. It is the oxide of iron that is credited for the pink color.

A support should have sufficient surface area so that the chosen amount of stationary phase can be deposited uniformly and not leave an exposure of active sites on its surface. Conversely, if excessive phase (above the upper coating limit of the support) is deposited on the support, phase may have a tendency to "puddle or pool" on a support particle and can even spread over to an adjacent particle, resulting in a decrease in column efficiency due to unfavorable mass transfer. In Figure 3.2 a series of scanning electron micrographs of 20% Carbowax 20M on 80/100-mesh Chromosorb W HP are illustrated. A photomicrograph of a nonhomogeneous deposition of phase is shown in Figure 3.2a, where a large amount of polymer distributed between two particles is visible in the left portion of the photograph. This packing ultimately yielded a column of low efficiency because of unfavorable mass transfer, as opposed to a higher column efficiency associated with a column packed with a more uniformly coated support (Figure 3.2b).

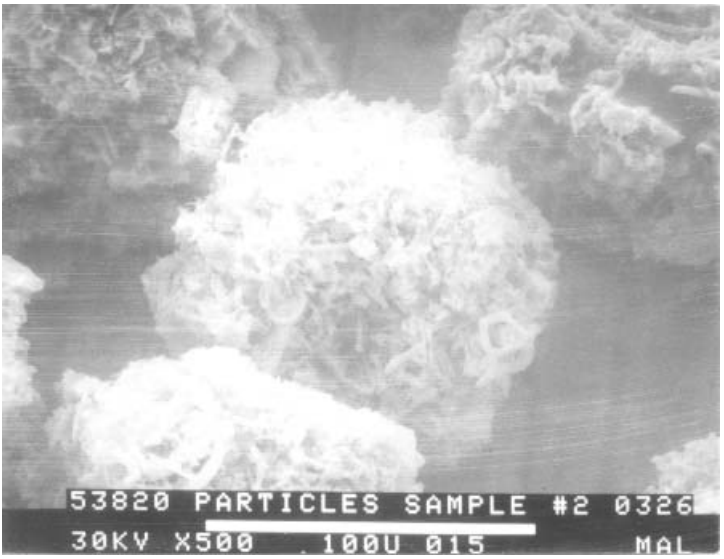
The pink-colored firebrick supports such as Chromosorb P and Gas Chrom R are very strong particulates that provide higher column plate numbers than most supports. Because of their high specific surface area, these supports can accommodate up to 30% loading of liquid phase, and their use is reserved for the analysis of non-polar species such as hydrocarbons. They must be deactivated, however, when employed for the analysis of polar compounds such as alcohols and amines. As a result, the white-colored filter aid supports of lower surface area (Chromosorb W, Gas Chrom Q and Supelcoport, to name several), are preferable although they are more fragile and permit a slightly lower maximum percent loading of about 25% by weight of liquid phase. The harder and improved support, Chromosorb G, is denser than the Chromosorb W but also exhibits a lower surface area and is used for the analysis of polar compounds. Chromosorb G, manufactured in a fashion similar to that for filter aid supports, is considerably more durable. Its maximum loading is 5% by weight.

It has been well established that the surface of the diatomites are covered with silanol (Si–OH) and siloxane (Si–O–Si) groups. The pink diatomite is more adsorptive than the white; this difference is due to the greater surface area per unit volume rather than in any fundamental surface characteristic. The pink diatomite is slightly acidic (pH 6−7), whereas the white diatomite is slightly basic (pH 8−10). Both types of diatomites have two sites for adsorption: (1) van der Waals sites and (2) hydrogen-bonding sites. Hydrogen-bonding sites are more important, and there are two different types for hydrogen bonding: *silanol groups*, which act as a proton donor, and the *siloxane group*, where the group acts as a proton acceptor. Thus, samples containing hydrogen bonds (e.g., water, alcohol, and amines) may show considerable tailing, whereas those compounds that hydrogen-bond to a lesser degree (e.g., ketones, esters) do not tail as much.

A support should ideally be inert and not interact with sample components in any way, otherwise a component may decompose on the column resulting in peak tailing or even disappearance of the peak in a chromatogram. The presence of active silanol groups (Si–OH functionalities) and metal ions constitute two types of active adsorptive sites on support materials. Polar analytes, acting as Lewis bases, can participate in hydrogen bonding with silanol sites and display



(*a*)



(*b*)

FIGURE 3.2 Scanning electron photomicrographs of (a) nonuniform coating of 20% Carbowax 20M on Chromosorb W HP, 80/100 mesh; note the stationary-phase "pooling" in left-hand portion of photograph and (b) a more uniform coating of Carbowax 20M. (Reference 7.)

peak tailing. The degree of tailing increases in the sequence hydrocarbons, ethers, esters, alcohols, carboxylic acids, and so on and also increases with decreasing concentration of a polar analyte. Treatment of the support with the most popular silylating reagent, dimethyldichlorosilane (DMDCS), converts silanol sites into silyl ether functionalities and generates a deactivated surface texture. Since this procedure is both critical and difficult (HCl is a product of the reaction), it is advisable to purchase DMDCS-deactivated support materials or column packings prepared with this chemically modified support material from a column manufacturer. A word of caution—the presence of moisture in the chromatographic system due to either impure carrier gas or water content in injected samples can hydrolyze silanized supports, reactivate them, as well as initiate degradation of many liquid phases.

Metal ions such as Fe^{3+} present on a diatomite support surface can likewise cause decomposition of both sample and stationary liquid phase. These ions can be considered Lewis acids that can also induce peak tailing of electron-dense analytes such as aromatics. These ions can be leached from the support surface by washing with hydrochloric acid followed by thorough rinsing to neutrality with deionized water of high quality. A Chromosorb support subjected to this treatment carries the suffix -AW; the untreated or non-acid-washed version of the same support is designated by the abbreviation -NAW. A support that is both acid-washed and deactivated with DMDCS is represented as -AW-DMDCS. The designation -HP is used for the classification of a support as high-performance grade, namely, the best available quality. A cross-reference of Chromosorb supports and the popular Gas Chrom series of supports is outlined as a function of type of diatomite and treatment in Table 3.1 and pertinent support properties are displayed in Table 3.2.

It has become the practice to refer to particle sizing of chromatographic supports in terms of the mesh range. For sieving of particles for chromatographic

TABLE 3.1 Cross-Reference of Solid Supports

Source	Acid Washed, DMDCS-Treated	Non-Acid-Washed	Acid-Washed
Firebrick	Chromosorb P AW-DMDCS	Chromosorb P NAW	Chromosorb P AW
	Gas Chrom RZ	Gas Chrom R	Gas Chrom RA
Celite filter aid	Chromosorb W AW-DMDCS	Chromosorb W NAW	Chromosorb W AW
	Chromosorb W HP[a]		
	Chromosorb G AW-DMDCS	Chromosorb G NAW	Chromosorb G AW
	Supelcoport[a]		
Other filter aid	Gas Chrom QII[a]		
	Gas Chrom Q (also base-washed, then silanized)		
	Gas Chrom Z	Gas Chrom S	Gas Chrom A

[a]High-performance support or best available grade of support.

Chromosorb, Gas Chrom, and Supelcoport are trademarks of Johns-Manville, Alltech, and Supelco, respectively.

TABLE 3.2 Properties of Selected Diatomaceous Earth Supports

Support	Packing Density (g/mL)	Surface Area (m^2/g)	Pore Volume (mL/g)	Maximum Liquid-Phase Loading (%)
Chromosorb P NAW	0.32–0.38	4–6	1.60	30
Chromosorb P AW	0.32–0.38	4–6		
Chromosorb P AW-DMDCS	0.32–0.38	4–6		
Chromosorb W AW	0.21–0.27	1.0–3.5	3.56	15
Chromosorb W HP	0.23	0.6–1.3		
Chromosorb G NAW	0.49	0.5	0.92	5
Chromosorb G AW-DMDCS	0.49	0.5		
Chromosorb G HP	0.49	0.4		

Source: Data obtained from References 8 and 9.

columns, both the Tyler standard screens and the U.S. standard series are frequently used. Tyler screens are identified by the actual number of meshes per linear inch. The U.S. sieves are identified by either micrometer (μm) designations or arbitrary numbers. Thus, a material referred to as 60/80-mesh means particles that will pass through a 60-mesh screen but not an 80-mesh screen. You may also see this written as $-60 + 80$ mesh. Particle size is much better expressed in micrometers; therefore, 60/80 mesh would correspond to 250–177 μm particle size range. Table 3.3 shows the conversion of column-packing particle sizes and also the relationship between mesh size, micrometers, millimeters, and inches. Table 3.4 shows the relationship between particle size and sieve size.

Lack of the proper amount of packing in a gas chromatographic column often is the source of a poor separation. How can one tell when a column is properly packed? The answer is twofold: by column performance (efficiency) and by peak symmetry. Many factors affect column performance; loosely packed columns generally are inefficient and are easily noticeable with glass chromatographic columns. A column that is too tightly packed gives excessive pressures drop or may even become completely plugged because the support particles have been broken and fines are present.

Small quantities of acids and bases may also be added to the stationary phase to cover or neutralize active sites on support. They usually have the same acid–base properties of the species being analyzed and are referred to as "tail reducers." Phosphoric acid–modified packings are effective for analyzing fatty acids and phenols; potassium hydroxide has been used with success for amines and other basic compounds.

An often overlooked parameter in the selection of a packed column is the packing density of the support material. Packing density can have a rather pronounced effect on retention data. The stationary phase is coated on a support on a weight percent basis, whereas the packing material is placed in the column on a volume basis. If the packing density of a support increases, then the total amount of stationary phase in the column increases, even if the loading percentage is

TABLE 3.3 Conversion Table of Column Packing Particles of Chromatographic Significance

Mesh Size	Micrometers	Millimeters	Inches
20	840	0.84	0.0328
30	590	0.59	0.0232
40	420	0.42	0.0164
50	297	0.29	0.0116
60	250	0.25	0.0097
70	210	0.21	0.0082
80	177	0.17	0.0069
100	149	0.14	0.0058
140	105	0.10	0.0041
200	74	0.07	0.0069
230	62	0.06	0.0024
270	53	0.05	0.0021
325	44	0.04	0.0017
400	37	0.03	0.0015
625	20	0.02	0.0008
1250	10	0.01	0.0004
2500	5	0.005	0.0002

TABLE 3.4 Relationship between Particle Size and Screen Openings

Sieve Size	Top Screen Openings (μm)	Bottom Screen Openings (μm)	Micrometer Spread
10/30	2000	590	1410
30/60	590	250	340
35/80	500	177	323
45/60	350	250	100
60/80	250	177	73
80/100	177	149	28
100/120	149	125	24
120/140	125	105	20
100/140	147	105	42

constant. Packing density varies among support materials (Table 3.2) and may even vary from batch to batch for a given type of support. Consider the following scenario. Two packings are prepared, 10% Carbowax 20M on Chromosorb G HP, the other Carbowax 20M on Chromosorb W HP, and each subsequently packed into glass columns of identical dimensions. The column containing the impregnated Chromosorb W HP will contain approximately twice as much stationary phase as the other column. Therefore, careful adherence should be paid to the

nature and properties of a support in order to generate meaningful retention data and compare separations.

3.5.1.2 Teflon Supports

Although diatomite supports are widely used support materials, analysis of corrosive or very polar substances requires even more inertness from the support. Halocarbon supports offer enhanced inertness, and a variety have been tried, including Fluoropak-80, Kel-F, Teflon, and other fluorocarbon materials. However, Chromosorb T, made from Teflon 6 powder, is perhaps the best material available because high column efficiencies can be obtained when it is coated with a stationary phase having high surface area such as polyethylene glycols. Chromosorb T has a surface area of 7–8 m^2/g, a packing density of 0.42 g/mL, an upper coating limit of 20%, and a rather low upper temperature limit of 250°C. Applications where this type of support is recommended are the analyses of water, acids, amines, HF, HCl, chlorosilanes, sulfur dioxide, and hydrazine. Difficulties in coating Chromosorb T and packing columns may be encountered as the material tends to develop static charges. This situation is minimized by using (1) plasticware in place of glass beakers, funnels, and other components; (2) chilling the support to 10°C prior to coating; and (3) also chilling the column before packing. References 12–16 yield further information for successful results with this support. However, preparation of columns containing Teflon-coated stationary phases is best performed by the column manufacturers.

The interested reader desiring further details about solid supports is urged to consult the comprehensive reviews of Ottenstein (10,11) and the benchmark book, *The Packed Column in Gas Chromatography,* written by Supina (12).

3.5.2 Adsorbents for GSC: Porous Polymers, Molecular Sieves, Carbonaceous Materials

Surface adsorption is the prevailing separation mechanism in gas–solid chromatography (GSC) while great care is taken to avoid this effect in gas-liquid chromatography (GLC). In GSC an uncoated adsorbent serves as the column packing, although special effects in selectivity by a mixed retention mechanism can be obtained by coating the adsorbent with a stationary phase. The latter case is an illustration of the mode, gas–liquid–solid chromatography (GLSC). Permanent gases and very volatile organic compounds can be analyzed by GSC as their volatility is problematic in GLC because their volatility causes rapid elution.

3.5.2.1 Porous Polymers

Porous polymers are the adsorbents of choice for most applications focusing on the analysis of gases, organics of low carbon number, acids, amines, and water (17,18). The presence of water is detrimental to gas–liquid chromatographic packings. Because water is eluted with symmetric band profiles on a

number of porous polymers, these adsorbents may be employed for the analyses of aqueous solutions and the determination of water in organic matrices. There are three separate product lines of commercially available porous polymers, namely, the Porapaks (Millipore Corp.), the Chromosorb Century Series (Johns-Manville), and HayeSep (Hayes Separation) polymers. Within each product line there are several members, each differing in chemical composition and, therefore, exhibiting unique selectivity, as may be observed in Table 3.5. On the other hand, some adsorbents are quite similar such as is the case with Porapak Q-Chromosorb 102 (both styrene–divinylbenzene copolymers) and HayeSep C-Chromosorb 104 (both acrylonitrile–divinylbenzene copolymers). We should expect to see in the future new polymers addressing old separation problems as was the case with the arrival of HayeSep A, which can resolve a mixture of nitrogen, oxygen, argon, and carbon monoxide at room temperature (19).

3.5.2.2 Molecular Sieves

These sorbents are also referred to as *zeolites*, which are synthetic alkali or alkaline-earth metal aluminum silicates and are utilized for the separation of hydrogen, oxygen, nitrogen, methane, and carbon monoxide. These substances are separated on molecular sieves because the pore size of the sieve matches their molecular diameter. There are two popular types of molecular sieves used in GSC, Molecular Sieve 5A (pore size of 5 Å with calcium as primary cation) and Molecular Sieve 13X (pore size of 13 Å with sodium as primary cation). At normal column temperatures, molecular sieves permanently adsorb carbon dioxide, which gradually degrades the O_2–N_2 resolution. The use of a silicagel precolumn that adsorbs carbon dioxide eliminates this problem. Molecular sieve columns must be conditioned at 300°C to remove residual moisture from the packing; otherwise the permanent gases elute too quickly, with little or no resolution, and coelution or reversal in elution order for CO methane may occur (20).

3.5.2.3 Carbonaceous Materials

Adsorbents containing carbon are commercially available in two forms: carbon molecular sieves and graphitized carbon blacks. The use of carbon molecular sieves as packings for GSC were first reported by Kaiser (21). They behave similarly to molecular sieves because their pore network is also in the angstrom range. Permanent gases and C1–C3 hydrocarbons may be separated on carbonaceous sieves such as Carbosphere and Carboxen.

Graphitized carbons play a dual role in GC. They are a nonspecific adsorbent in GSC having a surface area in the range of 10–1200 m^2/g. Adsorbents such as Carbopacks and Graphpacs may also serve as a support in GLC and in GLSC where unique selectivity is acquired and a separation is based on molecular

TABLE 3.5 Porous Polymeric Adsorbents for GSC

Adsorbent	Polymeric Composition or Polar Monomer (PM)	Maximum Temp (°C)	Applications
HayeSep A	DVB-EGDMA	165	Permanent gases, including hydrogen, nitrogen, oxygen, argon, CO, and NO at ambient temperature; can separate C2 hydrocarbons, hydrogen sulfide, and water at elevated temperatures
HayeSep B	DVB-PEI	190	C1 and C2 amines; trace amounts of ammonia and water
HayeSep C	ACN-DVB	250	Analysis of polar gases (HCN, ammonia, hydrogen sulfide) and water
HayeSep D	High purity DVB	290	Separation of CO and carbon dioxide from room air at ambient temperature; elutes acetylene before other C2 hydrocarbons; analyses of water and hydrogen sulfide
Porapak N	DVB-EVB-EGDMA	190	Separation of ammonia, carbon dioxide, water, and separation of 165 acetylene from other C2 hydrocarbons
HayeSep N	EGDMA (copolymer)		
Porapak P	Styrene-DVB	250	Separation of a wide variety of alcohols, glycols, and carbonyl analytes
HayeSep P	Styrene-DVB	250	
Porapak Q	EVB-DVB copolymer	250	Most widely used; separation of hydrocarbons, organic analytes in water, and oxides of nitrogen
HayeSep Q	DVB Polymer	275	
Porapak R	Vinyl pyrollidone (PM)	250	Separation of ethers and esters; separation of water from chlorine and HCl
HayeSep R		250	
Porapak S	Vinyl pyridine (PM)	250	Separation of normal and branched alcohols
HayeSep S	DVB-4-vinylpyridine	250	

TABLE 3.5 (*Continued*)

Adsorbent	Polymeric Composition or Polar Monomer (PM)	Maximum Temp (°C)	Applications
Porapak T	EGDMA (PM)	190	Highest-polarity Porapak; offers greatest water retention; determination of formaldehyde in water
HayeSep T	EGDMA Polymer	165	
Chromosorb 101	Styrene-DVB	275	Separation of fatty acids, alcohols, glycols, esters, ketones, aldehydes, and ethers and hydrocarbons.
102	Styrene-DVB	250	Separation of volatile organics and permanent gases; no peak tailing for water and alcohols
103	Cross-linked PS	275	Separation of basic compounds, such as amines and ammonia; useful for separation of amides, hydrazines, alcohols, aldehydes, and ketones
104	ACN-DVB	250	Nitriles, nitroparaffins, hydrogen sulfide, ammonia, sulfur dioxide, carbon dioxide, vinylidene chloride, vinyl chloride, trace water content in solvents
105	Crosslinked polyaromatic	250	Separation of aqueous solutions of formaldehyde, separation of acetylene from lower hydrocarbons and various classes of organics with boiling points up to 200°C
106	Crosslinked PS	225	Separation of C2–C5 alcohols; separation of C2–C5 fatty acids from corresponding alcohols
107	Crosslinked acrylic ester	225	Analysis of formaldehyde, sulfur gases, and various classes of compounds
108	Crosslinked acrylic	225	Separation of gases and polar species such as water, alcohols, aldehydes, ketones, glycols

Key: DVB—divinylbenzene; EGDMA—ethylene glycol dimethacrylate; PEI—polyethyleneimine; ACN—acrylonitrile; EVB—ethylvinylbenzene.

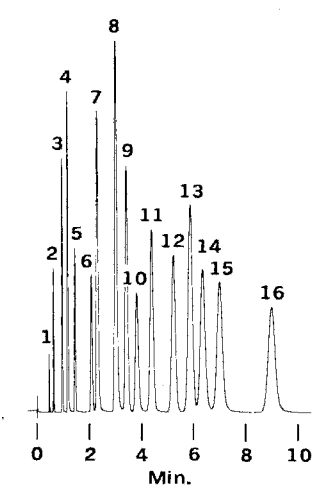
Source: Data obtained from References 8, 15, and 16.

geometry and polarizability considerations. Coated graphitized carbons can tolerate aqueous samples and have been used for the determination of water in glycols, acids, and amines by DiCorcia and co-workers (22–24). In the latter roles, since graphitized carbon has a nonpolar surface texture, it must be coated with a stationary phase for deactivation of its surface. The resulting packing reflects a separation that is a hybrid of gas–solid and gas–liquid mechanisms. Frequently, the packing is further modified by the addition of H_3PO_4 or KOH to reduce peak tailing for acidic and basic compounds, respectively. Separations of alcohols and amines are displayed in Figure 3.3. The USP (*United States Pharmacopoeia*) support designations specified in many gas chromatographic methods appear in Table 3.6.

TABLE 3.6 USP Designations of Popular Supports and Adsorbents

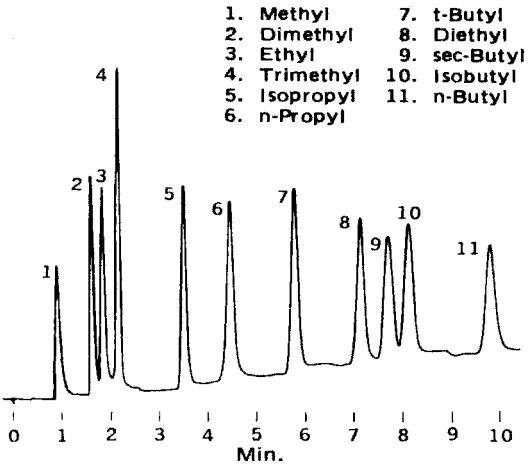
USP Nomenclature	USP Support Description
S1A	Siliceous earth (Chromosorb W; see method 1 for details on treatment)
S1AB	Siliceous earth, treated as S1 and both acid- and base-washed
S1C	Crushed firebrick, calcined or burned with a clay binder above 900°C, acid-washed, may be silanized (i.e., Chromosorb P)
S1NS	Untreated siliceous earth (i.e., Chromosorb W)
S2	Styrene–divinylbenzene copolymer with nominal surface area of <50 m^2/g and an average pore diameter of 0.3–0.4 μm
S3	Styrene–divinylbenzene copolymer with nominal surface area of 500–600 m^2/g and an average pore diameter of 0.0075 μm
S4	Styrene–divinylbenzene copolymer with aromatic –O and –N groups having a nominal surface area of 400–600 m^2/g and an average pore diameter of 0.0076 μm
S5	High-molecular-weight tetrafluoroethylene polymer, 40/60-mesh
S6	Styrene–divinylbenzene copolymer having a nominal surface area of 250–350 m^2/g and an average pore diameter of 0.0091 μm
S7	Graphitized carbon having a nominal surface area of 12 m^2/g
S8	Copolymer of 4-vinylpyridine and styrene–divinylbenzene
S9	Porous polymer based on 2,6-diphenyl-*p*-phenyl oxide
S10	Highly crosslinked copolymer of acrylonitrile and divinylbenzene
S11	Graphitized carbon having a nominal surface area of 100 m^2/g, modified with small amounts of petrolatum and poly(ethylene glycol) compound
S12	Graphitized carbon having a nominal surface area of 100 m^2/g

Source: *USP Column Cross-reference Chart*, Restek Corporation.



80/100 Carbopack C/0.2% Carbowax 1500, 6′ x 2mm ID glass, Col. Temp.: 135°C, Flow Rate: 20mL/min., N_2, Det.: FID, Sample Size: 0.02μL.

(*a*)



60/80 Carbopack B/4% Carbowax 20M/0.8% KOH, 6′ x 2mm ID glass, Col. Temp.: 90°C to 150°C @ 4°C/min., Flow Rate: 20mL/min., N_2, Det.: FID, Sens.: 4 x 10^{-11} AFS, Sample: 0.5μL, 100ppm each amine in water.

(*b*)

FIGURE 3.3 Separation of C1–C5 alcohols (a) and aliphatic amines (b) on graphitized carbon. (Reproduced from Reference 20: W. A. Supina, in *Modern Practice of Gas Chromatography*, 2nd ed., R. L. Grob, ed., copyright 1985, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

3.6 STATIONARY PHASES

3.6.1 Requirements of a Stationary Phase

An ideal stationary liquid phase for GLC should exhibit selectivity and differential solubility of components to be separated and a wide operating temperature range. A phase should be chemically stable and have a low vapor pressure at elevated column temperatures. A minimum temperature limit near ambient temperature, where the liquid phase still exists as a liquid and not as a solid, is desirable for separations at or near room temperature and eliminates a gas–solid adsorption mechanism prevailing such as with Carbowax 20M below 60°C. In choosing a liquid phase some fundamental criteria must be considered:

1. Is the liquid phase selective toward the components to be separated?
2. Will there be any irreversible reactions between the liquid phase and the components of the mixture to be separated?
3. Does the liquid phase have a low vapor pressure at the operating temperature? Is it thermally stable?

Let us consider some of the information available to answer these questions, although we are not now attempting to develop a pattern for selection of the proper liquid substrate; this will be discussed later in the chapter. The vapor pressure of the liquid phase should be less than 0.1 Torr at the operating temperature of the column. This value can change depending on the detector used, since bleed from the liquid phase will cause noise and elevate background signal and thus decrease sensitivity. Information from a plot of vapor pressure versus temperature is not always completely informative nor practical sometimes because adsorption of the liquid phase on the solid support results in decrease in the actual vapor pressure of the liquid phase. Other than its effect on the detector noise, liquid-phase bleed may interfere with analytical results and determine the life of the column. Also, some supports may have a catalytic effect to decompose the liquid phase, thereby reducing its life in the column. Contaminants in the carrier gas (e.g., O_2) also may cause premature fatigue of a liquid phase. The effect of impurities in gases used in GC is treated in Chapter 10. Two other properties of the liquid phase to be considered are viscosity and wetting ability. Ideally, liquid phases should have low viscosity and high wetting ability (ability to form a uniform film on the solid support or column wall).

It is uninformative to refer to a liquid phase as being selective, since all liquid phases are selective to varying degrees. “Selectivity” refers to the relative retention of two components and gives no information regarding the mechanism of separation. Most separations depend on boiling point difference, variations in molecular weights of the components, and the structure of the components.

The relative volatility or separation factor α depends on the interactions of the solute and the liquid phase, that is, van der Waals cohesive forces. These cohesive forces may be divided into three types:

1. *London Dispersion Forces.* These are due to the attraction of dipoles that arise from the arrangement of the elementary charges. Dispersion forces act

between all molecular types and especially in the separation of nonpolar substances (e.g., saturated hydrocarbons).

2. *Debye Induction Forces*. These forces result from interaction between permanent and induced dipoles.
3. *Keesom Orientation Forces*. These forces result from the interaction of two permanent dipoles, of which the hydrogen bond is the most important. Hydrogen bonds are stronger than dispersion or inductive forces. If the two components have the same vapor pressure, separation can be achieved on the basis of several properties. These properties are (in the order of their ease of separation) (1) difference in the functional groups, (2) isomers with polar functional groups, and (3) isomers with no functional groups.

In the selection of a stationary phase a compromise between theory and practice must be reconciled. For example, theory dictates that a stationary phase of low viscosity or fluid in texture is preferable over a chemically equivalent, more viscous gum phase, as may be ascertained from the contribution of D_l, the solute diffusivity in the stationary phase, appearing in Equation 2.44. However, this same fluid possessing a lower molecular weight or weight distribution, if polymeric in nature, will typically have poorer thermal stability and a lower maximum operating temperature. Although unfavorable from the viewpoint of mass transfer in the van Deemter expression, practical considerations may favor the gum for separations requiring high column temperatures. Equation 2.44 indicates that a higher column efficiency is obtained with a column containing a low percent loading of stationary phase compared to a same column packed with a higher phase loading. But in practice, the deposition of a thin coating of stationary phase on a support may yield insufficient coverage of the active sites on the surface of the support, resulting in peak tailing, and reestablish a need for a higher percent of stationary phase loading. Note in Figure 3.4b the peak tailing of the n-alkanes on a lightly loaded packing (less than 3 percent OV-101 on Chromosorb W HP) and the elimination of tailing with a heavier coating of stationary phase (Figure 3.4a).

Separations in GLC are the resultant of selective solute–stationary-phase interactions and differences in the vapor pressure of solutes. The main forces that are responsible for solute interaction with a stationary phase are dispersion, induction, orientation and donor–acceptor interactions (25–27), the sum of which serves as a measure of the "polarity" of the stationary toward the solute. Selectivity, on the other hand, may be viewed in terms of the magnitude of the individual energies of interaction. In GLC, the selectivity of a column governs band spacing or the degree to which peak maxima are separated. The following parameters influence selectivity:

1. The nature of the stationary phase
2. The concentration of the stationary phase
3. Column temperature
4. The choice and pretreatment of solid support or adsorbent

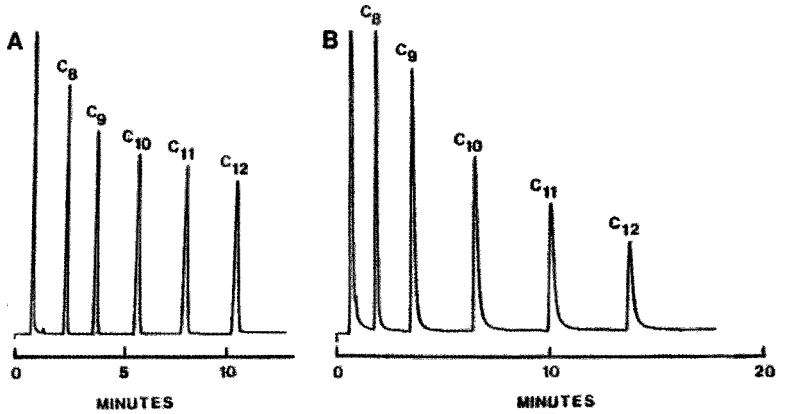


FIGURE 3.4 Chromatograms of *n*-alkanes on (a) 6-ft glass column, 2 mm i.d. containing 20% OV-101 on 80/100-mesh Chromosorb W HP, column conditions 100–175°C at 6°C/min; (b) same as in (a) but with 3% OV-101 on same support, column conditions 50–120°C at 4°C/min; flowrate 25 mL/min He. Det: FID. (Reference 7.)

Differences in selectivity are significant because they permit the separation of solutes of similar or even the same polarity by a selective stationary phase.

In the early practice of gas chromatography, the concept of polarity and even the requirements for a stationary phase were not clearly understood. There was a proliferation of liquid phases encompassing (1) those that had marginal gas chromatographic properties such as Nujol, glycerol, diglycerol, and Tide, the laundry detergent; (2) those that were industrial-grade lubricants of variable composition, such as the Apiezon greases and Ucon oils; and (3) an abundance of phases that just simply duplicated the chromatographic behavior of others. In retrospect, the vast array of stationary phases can probably be attributed to the compensation for the inefficiency of a packed column by achieving some acceptable degree of selectivity for the resolution of two solutes (Equation 3.6). Conversely, the high efficiency of a capillary column allows the availability of a relatively few stationary phases, each differing in selectivity to achieve any required resolution.

The stationary phases requirements of selectivity and higher thermal stability then became more clearly defined; the process of stationary-phase selection and classification became logical after the studies of McReynolds (28) and Rohrschneider (29,30) were published, both of which were based on the retention index (31). The Kovats retention index procedure and McReynolds constants are discussed in detail in the following section. Kovats retention indices today remain a widely used technique for reporting retention data, while every stationary phase developed for packed and capillary GC has been characterized by generation of its McReynolds constants.

3.6.2 Kovats Retention Indices

This universal approach solved the problems pertaining to the use, comparison, and characterization of gas chromatographic retention data. The reporting

of retention data as absolute retention time t_R is meaningless because virtually every chromatographic parameter and any related experimental fluctuation affect a retention time measurement. The use of relative retention data ($\alpha = t'_{R2}/t'_{R1}$) offered some improvement but the lack of a universal standard suitable for wide temperature range on stationary phases of different polarities has discouraged its utilization. In the Kovats approach, the retention index I of an n-alkane is assigned a value equal to 100 times its carbon number. Thus, for example, the I values of n-octane, n-decane, and n-dodecane are equal to 800, 1000 and 1200, respectively, by definition and are applicable on any column, packed or capillary, any liquid phase and independent of every chromatographic condition, including column temperature. However, for all other compounds, the chromatographic conditions such as the stationary phase, its concentration, support, and column temperature for packed columns must be specified. Since retention indices are also the preferred method for reporting retention data with capillary columns, the stationary phase, film thickness, and column temperature likewise have to be specified for compounds other than n-alkanes; otherwise the I values are meaningless.

An I value of a component can be determined by spiking a mixture of n-alkanes with the component(s) of interest and chromatographing the resulting mixture under the specified conditions. A plot of log-adjusted retention time versus retention index is generated, and the retention index of the solute under consideration is determined by extrapolation, as depicted in Figure 3.5 for

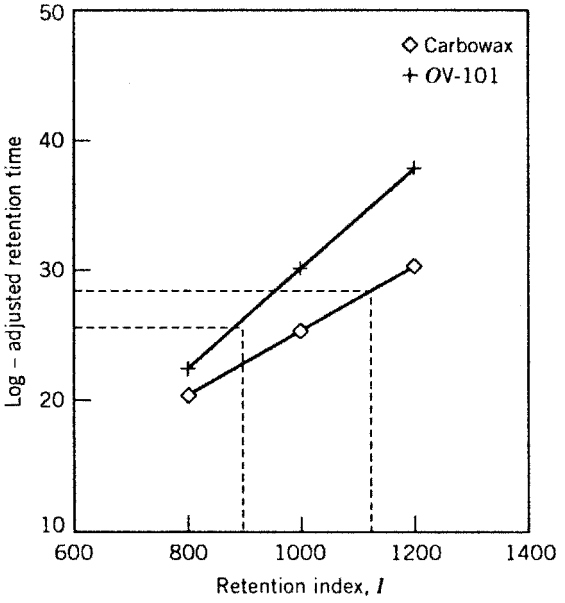


FIGURE 3.5 Plot of logarithm-adjusted retention time versus Kovats retention index: isoamyl acetate at 120°C.

isoamyl acetate. The selectivity of a particular stationary phase can be established by comparing the I values of a solute on a nonpolar phase such as squalane or OV-101 ($I = 872$) with the corresponding value of I of 1128 associated with a more polar column containing Carbowax 20M, for example. This difference of 256 units indicates the greater retention produced by the Carbowax 20M column. More specifically, isoamyl acetate elutes between n-C11 and n-C12 on a Carbowax 20M but more rapidly on OV-101 where it elutes after n-octane.

Alternatively, the retention index of an analyte at an isothermal column temperature can be calculated from the following equation:

$$I_x = \frac{100Z + 100[\log t'_{R,x} - \log t'_{R,z}]}{\log t'_{R,z+1} - \log t'_{R,z}} \tag{3.1}$$

where $t'_{R,x}$ is the adjusted retention time of the component under consideration, $t'_{R,z}$ is the adjusted retention time of the n-alkane eluting before it, $t'_{R,z+1}$ is the adjusted retention time of the n-alkane eluting after it, and Z is the carbon number of the n-alkane having retention $t'_{R,z}$. For temperature-programmed runs, the adjusted retention times in Equation 3.1 are replaced by the appropriate elution temperatures in degrees Kelvin. An I value computed by Equation 3.1 is strongly recommended because it is inherently more accurate than that obtained by the graphical approach.

Retention indices normalize instrumental variables in gas chromatographs, allowing retention data generated on different systems to be compared. For example, isoamyl acetate with a retention index of 1128 will elute between n-C11 and n-C12 under the same chromatographic conditions. Retention indices are also very helpful in comparing relative elution orders of series of analytes on a specific column at a given temperature and for comparing selective behavior of two or more columns.

McReynolds has tabulated retention indices for a large number of compounds on various liquid phases (32); an excellent review of the retention index system has been prepared by Ettre (33). The postrun calculation of retention indices is greatly facilitated by using a reporting integrator or a data acquisition system. Consistent with the growing trend of computer assistance in gas chromatography is the availability of retention index libraries for drugs and pharmaceuticals, organic volatiles, pesticides, herbicides and PCBs of environmental significance, methyl esters of fatty acids, food and flavor volatiles, solvents, and chemicals (34).

3.6.3 McReynolds Classification of Stationary Phases

The most widely used system of classifying liquid phases is the McReynolds system (28) and has been employed to characterize virtually every stationary phase. McReynolds selected 10 probe solutes of different functionality, each designated to measure a specific interaction with a liquid phase. He analyzed these probe solutes and measured their I values on over 200 phases, including squalane,

which served as a reference liquid phase under the same chromatographic conditions. A similar approach was previously implemented by Rohrschneider (29,30) with five probes. In Table 3.7 the probes used in both approaches and their function are listed. McReynolds calculated for each probe, a ΔI value, where

$$\Delta I = I_{\text{liquid phase}} - I_{\text{squalane}}$$

As the difference in the retention index for a probe on a given liquid phase and squalane increases, the degree of specific interaction associated with that probe increases. The cumulative effect, when summed for each of the 10 probes, is a measure of overall "polarity" of the stationary phase. In a tabulation of McReynolds constants, the first five probes usually appear and are represented by the symbols X', Y', Z', U', S'. Each probe is assigned a value of zero with squalane as reference liquid phase.

There were several significant consequences resulting from these classification procedures. Phases that have identical chromatographic behavior also have identical constants. In this case the selection of a stationary phase could be based on a consideration such as thermal stability, lower viscosity, cost, or availability. McReynolds constants of the more popular stationary phases for packed column GC are listed in Table 3.8. Note that the DC-200 (a silicone oil of low viscosity) and OV-101 or SE-30 (a dimethylpolysiloxane) have nearly identical

TABLE 3.7 Probes Used in McReynolds and Rohrschneider Classifications of Liquid Phases

Symbol	McReynolds Probe	Rohrschneider Probe	Measured Interaction
X′	Benzene	Benzene	Electron density for aromatic and olefinic hydrocarbons
Y′	*n*-Butanol	Ethanol	Proton donor and proton acceptor capabilities (alcohols, nitriles)
Z′	2-Pentanone	2-Butanone	Proton acceptor interaction (ketones, ethers, aldehydes, esters)
U′	Nitropropane	Nitromethane	Dipole interactions
S′	Pyridine	Pyridine	Strong proton acceptor interaction
H′	2-Methyl-2-pentanol	—	Substituted alcohol interaction similar to n-butanol
J′	Iodobutane	—	Polar alkane interactions
K′	2-Octyne	—	Unsaturated hydrocarbon interaction similar to benzene
L′	1,4-Dioxane	—	Proton acceptor interaction
M′	*cis*-Hydrindane	—	Dispersion interaction

TABLE 3.8 McReynolds Constants and Cross-Reference of Commonly Used Stationary Phases

Phase	Temperature (°C)	Chemical Nature	X′	Y′	Z′	U′	S′	Phases of Similar Structure
Squalane	20/100	Cycloparaffin	0	0	0	0	0	
		Polysiloxanes						
DC 200	0/200	Dimethylsilicone	16	57	45	66	43	SP-2100, SE-30, OV-101, OV-1
DC-710	5/250	Phenylmethylsilicone	107	149	153	228	190	OV-11
SE-30	50/300	Dimethyl	15	53	44	64	41	SP-2100, OV-101, OV-1
SE-54	50/300	5% phenyl, 1% vinyl	33	72	66	99	67	
OV-1	100/350	Dimethyl (gum)	16	55	44	65	42	SP-2100
OV-3	0/350	10% phenylphenyl-methyldimethyl	44	86	81	124	88	
OV-7	0/350	20% phenylphenyl-methyldimethyl	69	113	111	171	128	
OV-11	0/350	35% phenylphenyl-methyldimethyl	102	142	145	219	178	DC-710
OV-17	0/375	50% phenyl, 50% methyl	119	158	162	243	202	SP-2250
OV-22	0/350	65% phenylphenyl-methyldiphenyl	160	188	191	283	253	
OV-25	0/350	75% phenylphenyl-methyldiphenyl	178	204	208	305	280	

TABLE 3.8 (*Continued*)

Phase	Temperature (°C)	Chemical Nature	X′	Y′	Z′	U′	S′	Phases of Similar Structure
OV-61	0/350	33% phenyldiphenyl-dimethyl	101	143	142	213	174	
OV-73	0/325	5.5% phenyldiphenyl-dimethyl (gum)	40	86	76	114	85	
OV-101	0/350	Dimethyl (fluid)	17	57	45	67	43	SP-2100, SE-30, OV-1
OV-105	0/275	Cyanopropyl-methyldimethyl	36	108	93	139	86	
OV-202	0/275	Trifluoropropyl-methyl (fluid)	146	238	358	468	310	
OV-210	0/275	Trifluoropropyl-methyl (fluid)	146	238	358	468	310	SP-2401
OV-215	0/275	Trifluoropropyl-methyl (gum)	149	240	363	478	315	
OV-225	0/265	Cyanopropyl-methylphenylmethyl	228	369	338	492	386	SP-2300, Silar 5 CP
OV-275	25/275	Dicyanoallyl	629	872	763	1106	849	SP-2340
OV-330	0/250	Phenyl silicone-Carbowax copolymer	222	391	273	417	368	
OV-351	50/270	Carbowax-nitrotereph-thalic acid polymer	335	552	382	583	540	SP-1000

OV-1701	0/250	14% cyanopropylphenyl	67	170	153	228	171	
Silar 5 CP	0/250	50% cyanopropyl–50% phenyl	319	495	446	637	531	SP-2300, OV-225
Silar 10 CP	0/250	100% cyanopropyl	520	757	660	942	800	SP-2340
SP-2100	0/350	Methyl	17	57	45	67	43	SE-30, OV-101, OV-1
SP-2250	0/375	50% phenyl	119	158	162	243	202	OV-17
SP-2300	20/275	50% cyanopropyl	316	495	446	637	530	OV-225
SP-2310	25/275	55% cyanopropyl	440	637	605	840	670	
SP-2330	25/275	90% cyanopropyl	490	725	630	913	778	
SP-2340	25/275	100% cyanopropyl	520	757	659	942	800	Silar 10 CP
SP-2401	0/275	Trifluoropropyl	146	238	358	468	310	OV-210
		Nonsilicone Phases						
Apiezon L	50/300	Hydrocarbon grease	32	22	15	32	42	
Carbowax 20M	60/225	Poly(ethylene glycol)	322	536	368	572	510	Superox 4, Superox 20M
DEGS	20/200	Di(ethylene glycol) succinate	496	746	590	837	835	
TCEP	0/175	1,2,3-Tris(2-cyanoethoxy) propane	594	857	759	1031	917	
FFAP	50/250	Free fatty acid phase	340	580	397	602	627	OV-351

Source: Data obtained from References 8, 28, and 35.

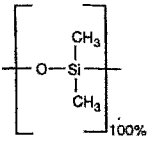
constants but also observe that these two polysiloxanes have a more favorable higher temperature limit. Comparisons of this type curtailed the proliferation of phases, eliminated the duplication of phases and simplified column selection. Many phases quickly became obsolete and were replaced by a phase having identical constants but of higher thermal stability such as a polysiloxane phase. Today polysiloxane-type phases are the most commonly used stationary phases for both packed-column (and capillary-column) separations because they exhibit excellent thermal stability, have favorable solute diffusivities and are available in a wide range of polarities. They will be discussed in greater detail in Part 3 of this chapter.

There likewise was an impetus to consolidate the number of stationary phases in use during the mid-1970s. In 1973 Leary et al. (36) reported the application of a statistical nearest-neighbor technique to the 226 stationary phases in the McReynolds study and suggested that just 12 phases could replace the 226. The majority of these 12 phases appear in Table 3.8. Delley and Friedrich found that four phases, OV-101, OV-17, OV-225, and Carbowax 20M, could provide satisfactory gas chromatographic analysis for 80% of a wide variety of organic compounds (37). Hawkes et al. (38) reported the findings of a committee effort on this subject and recommended a condensed list of six preferred stationary phases on which almost all gas–liquid chromatographic analysis can be performed: (1) a dimethylpolysiloxane (e.g., OV-101, SE-30, SP-210), (2) a 50% phenylpolysiloxane (OV-17, SP-2250), (3) poly(ethylene glycol) of molecular weight (MW) >4000 (Carbowax), (4) DEGS, (5) a 3-cyanopropylpolysiloxane (Silar 10 C, SP-2340), and (6) a trifluoropropylpolysiloxane (OV-210, SP-2401). Chemical structures of the more popular polysiloxanes used as stationary phases are illustrated in Figure 3.6.

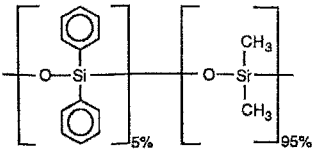
Another feature of the McReynolds constants is guidance in the selection of a column that will separate compounds with different functional groups, such as ketones from alcohols, ethers from olefins, and esters from nitriles. If an analyst wishes a column to elute an ester after an alcohol, the stationary phase should have a larger Z' value with respect to its Y' value. In the same fashion, a stationary should exhibit a larger Y' value with respect to Z' if an ether is to elute before an alcohol. The appendixes in Reference 12 list McReynolds constants in order of increasing ΔI for each probe in successive tables that are handy and greatly facilitate the column selection process.

3.6.4 Evaluation of Column Operation

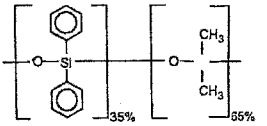
Several parameters can be used to evaluate operation of a column and to obtain information about a specific system. Using the principles underlying plate theory and others discussed in Section 2.3.1, plotting the concentration of solute (in percent) against volume of mobile phase or number of plate volumes for the 10th, 20th, and 50th plates in the column, we will obtain a plot as shown in Figure 3.7a. Improved separation of component peaks is possible for columns that have a larger plate number. Similar information is obtained if we plot concentration



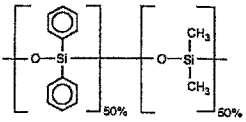
Poly(dimethylsiloxane)



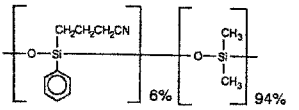
Poly(5%-diphenyl-95%-dimethylsiloxane)



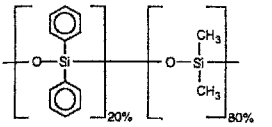
Poly(35%-diphenyl-65%-dimethylsiloxane)



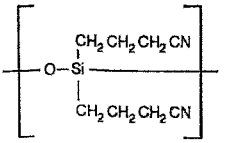
Poly(50%-diphenyl-50 %-dimethylsiloxane)



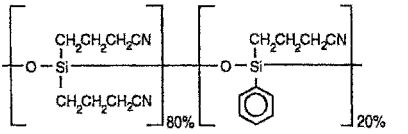
Poly(6%-cyanopropylphenyl-94%-dimethylsiloxane)



Poly(80%-diphenyl-50 %-dimethylsiloxane)



Poly(biscyanopropylsiloxane)



Poly(80 %-biscyanopropyl-20%-cyanopropylphenylsiloxane)

FIGURE 3.6 Chemical structures of popular polysiloxanes.

of solute (percent of total) versus plate number. Figure 3.7b shows the band positions after 50, 100, and 200 equilibrations with the mobile phase.

An ideal gas chromatographic column is considered to have high resolving power, high speed of operation, and high capacity. One of these factors can be improved usually at the expense of another. Sometime we may be able to achieve two of the three if we are fortunate. Thus, a number of column parameters must be discussed to enable us to arrive at an efficient operation of a column. We now consider several of these parameters and illustrate with appropriate relationships.

3.6.4.1 Column Efficiency

Two methods are available for expressing the efficiency of a column in terms of HETP: measurement of the peak width (Figure 2.18) at (1) the baseline (Equation 2.9) and (2) half-height (Equation 2.11). In determining N, we assume that the detector signal changes linearly with concentration. If it does not, N

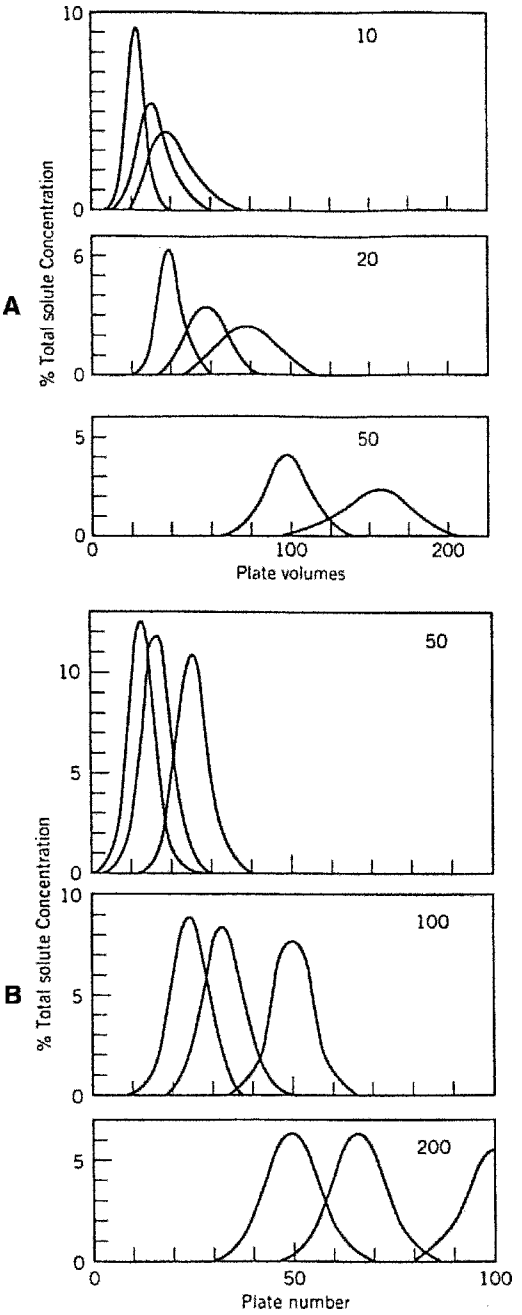


FIGURE 3.7 (a) Elution peaks for three solutes from various plate columns (top, 10 plates; middle, 20 plates; bottom, 50 plates); (b) plate position of components after variable number of equilibrations (top, 50 equilibrations; middle, 100 equilibrations; bottom, 200 equilibrations).

cannot measure column efficiency precisely. If Equation 2.9 or 2.11 is used to evaluate peaks that are not symmetric, positive deviations of 10–20% may result. Since N depends on column operating conditions, these should be stated when efficiency is determined. There are several ways by which one may calculate column efficiency other than the two equations shown (Equations 2.9 and 2.11). Figure 3.8 and Table 3.9 illustrate other ways in which this information may be obtained.

3.6.4.2 Effective Number of Theoretical Plates

The term "effective number of plates" N_{eff} was introduced to characterize open tubular columns. In this relationship adjusted retention volume V'_{R}, in lieu of total retention volume V_{R}, is used to determine plate number:

$$N_{\text{eff}} = 16\left(\frac{V'_{\text{R}}}{w_{\text{b}}}\right)^2 = 16\left(\frac{t'_{\text{R}}}{w_{\text{b}}}\right)^2 \tag{3.2}$$

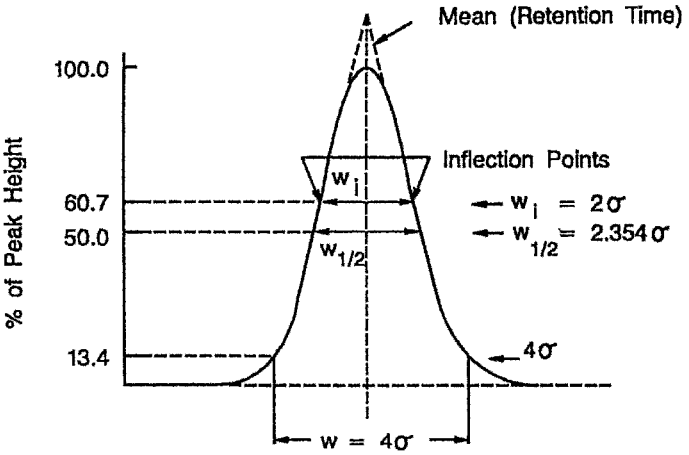


FIGURE 3.8 Pertinent points on a chromatographic band for calculation of column efficiency.

TABLE 3.9 Calculation of Column Efficiency from Chromatograms

Standard Deviation Terms	Measurements	Plate Number $N =$
$A/h(2\pi)^{1/2}$	t_{R} and band area A and height h	$2\pi(t_{\text{R}}h/A)^2$
$W_i/2$	t_{R} and width at inflection points $(0.607h)w_i$	$4(t_{\text{R}}/w_i)^2$
$w_{\text{h}}/(8\ \ln\ 2)^{1/2}$	t_{R} and width at half-height w_{h}	$5.55(t_{\text{R}}/w_{\text{h}})^2$
$w_{\text{b}}/4$	t_{R} and baseline width w_{b}	$16(t_{\text{R}}/w_{\text{h}})^2$

This N_{eff} value is useful for comparing a packed and an open tubular column or two similar columns when both are used for the same separation. Open tubular columns generally have a larger number of theoretical plates. One can translate regular number of plates N to effective number of plates N_{eff} by the expression

$$N_{\text{eff}} = N\left(\frac{k}{1+k}\right)^2 \tag{3.3}$$

as well as the plate height to the effective plate height:

$$H_{\text{eff}} = H\left(\frac{1+k}{k}\right)^2 \tag{3.4}$$

Similarly, the number of theoretical plates per unit time can be calculated:

$$\frac{N}{t_{\text{R}}} = \frac{\overline{u}(k)^2}{t_{\text{R}}(1+k)^2} \tag{3.5}$$

where $\overline{u}$ is the average linear gas velocity. This relationship accounts for characteristic column parameters, thus offering a way to compare different-type columns.

3.6.4.3 Resolution

The separation of two components as the peaks appear on the chromatogram (see Figure 2.18) is characterized by

$$R_{\text{s}} = \frac{2\Delta t'_{\text{R}}}{w_{\text{b1}} + w_{\text{b2}}} \tag{3.6}$$

where $\Delta t'_{\text{R}} = t'_{\text{R2}} - t'_{\text{R1}}$. If the peak widths are equal, that is, $w_{\text{b1}} = w_{\text{b2}}$, Equation 3.6 may be rewritten

$$R_{\text{s}} = \frac{\Delta t'_{\text{R}}}{w_{\text{b}}} \tag{3.7}$$

The two peaks will touch at the baseline when $\Delta t'_{\text{R}}$ is equal to $4s$:

$$t'_{\text{R2}} - t'_{\text{R1}} = \Delta t'_{\text{R}} \tag{3.8}$$

If two peaks are separated by a distance $4s$, then $R_{\text{s}} = 1$. If the peaks are separated by a $6s$, then $R_{\text{s}} = 1.5$.

Resolution also may be expressed in terms of retention indices of two components:

$$R_{\text{s}} = \frac{I_2 - I_1}{w_{\text{h}} f} \tag{3.9}$$

where f is the correction factor (1.699) because $4s = w_{\text{b}} = 1.699 w_{\text{h}}$.

A more useable expression for resolution is

$$R_s = \frac{1}{4}(N)^{1/2}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{1+k}\right) \tag{3.10}$$

where N and k refer to the later-eluting compound of the pair. Since α and k are constant for a given column (under isothermal conditions), resolution will be dependent on the number of theoretical plates N. The k term generally increases with a temperature decrease as does α but to a lesser extent. The result is that at low temperatures one finds that fewer theoretical plates or a shorter column are required for the same separation.

3.6.4.4 Required Plate Number

If the retention factor k and the separation factor α are known, the required number of plates (n_{ne}) can be calculated for the separation of two components. (The k value refers to the more readily sorbed component.) Thus

$$n_{ne} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1+k}{k}\right)^2 \tag{3.11}$$

The R_s value is set at the 6s level or 1.5. In terms of the required effective number of plates, Equation 3.11 would be

$$N_{eff} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2 \tag{3.12}$$

Taking into account the phase β ratio, we can write Equation 3.11 as

$$n_{ne} = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{\beta}{k_2} + 1\right)^2 \tag{3.13}$$

Equations 3.11 and 3.13 illustrate that the required number of plates will depend on the partition characteristics of the column and the relative volatility of the two components, that is, on K and β. Table 3.10 gives the values of the last term of Equation 3.13 for various values of k. These data suggest a few interesting conclusions. If $k < 5$, the plate numbers are controlled mainly by column parameters; if $k > 5$, the plate numbers are controlled by relative volatility of components. The data also illustrate that k values greater than 20 cause the theoretical number of plates N and effective number of plates N_{eff} to be the same order of magnitude:

$$N \simeq N_{eff} \tag{3.14}$$

TABLE 3.10 Values for Last Term of Equation 3.11

k	0.25	0.5	1.0	5.0	10	20	50	100
$(1 + k/k)^2$	25	9	4	1.44	1.21	1.11	1.04	1.02

The relationship in Equation 3.11 also can be used to determine the length of column necessary for a separation L_{ne}. We know that $N = L/H$; thus

$$L_{ne} = 16R_s^2 H \left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{1+k}{k}\right)^2 \tag{3.15}$$

Unfortunately, Equation 3.15 is of little practical importance because the H value for the more readily sorbed component must be known but is not readily available from independent data.

Let us give some examples from the use of Equation 3.11. Table 3.11 gives the number of theoretical plates for various values of α and k, assuming R_s to be at 6s (1.5). Using data in Table 3.11 and Equation 3.11, we can make an approximate comparison between packed and open tubular columns. As a first approximation, β values of packed columns are 5–30 and for open tubular columns, 100–1000—thus a 10–100-fold difference in k. Examination of the data in Table 3.11 shows that when $\alpha = 1.05$ and $k = 5.0$ we would need 22,861 plates in a packed column, which would correspond to an open tubular column with $k = 0.5$ having 142,884 plates. Although a greater number of plates is predicted for the open tubular column, this is relatively easy to attain because longer columns of this type have high permeability and smaller pressure drop than the packed columns.

3.6.4.5 Separation Factor

The reader will recall that the separation factor α in Section 1.2 is the same as the relative volatility term used in distillation theory. In 1959 Purnell (39,40) introduced another separation factor term (S) to describe the efficiency of a column.

TABLE 3.11 Number of Theoretical Plates for Values of α and k (R_s at 6σ = 1.5)

k	α: 1.05	1.10	1.50	2.00	3.00
0.1	1,920,996	527,076	39,204	17,424	9,801
0.2	571,536	156,816	11,664	5,184	2,916
0.5	142,884	39,204	2,916	1,296	729
1.0	63,504	17,424	1,296	576	324
2.0	35,519	9,801	729	324	182
5.0	22,861	6,273	467	207	117
8.0	20,004	5,489	408	181	102
10.0	19,210	5,271	392	173	97

It can be used very conveniently to describe efficiency of open tubular columns:

$$S = 16\left(\frac{V'_R}{w_b}\right)^2 = 16\left(\frac{t'_R}{w_b}\right)^2 \tag{3.16}$$

where V'_R and t'_R = adjusted retention volume and adjusted retention time, respectively. Equation 3.16 may be written as a thermodynamic quantity that is characteristic of the separation but independent of the column. In this form we assume resolution R_s at the 6s level or having a value of 1.5. Therefore, from Equation 3.12, we obtain

$$S = 36\left(\frac{\alpha}{\alpha - 1}\right)^2 \tag{3.17}$$

3.6.4.6 Separation Number

We also can calculate a separation number SN or Trennzahl abbreviated as TZ as another way of describing column efficiency (41). By separation number we mean the number of possible peaks that appear between two n-paraffin peaks with consecutive carbon numbers. It may be calculated by

$$\mathrm{SN} = \left[\frac{t_{R_2} - t_{R_1}}{(w_h)_1 + (w_h)_2}\right] - 1 \tag{3.18}$$

This equation may be used to characterize capillary columns or for application of programmed pressure or temperature conditions for packed columns. This concept is depicted in Figure 3.9.

3.6.4.7 Analysis Time

If possible, we like to perform the chromatographic separation in minimum time. Time is important in analysis but it is particularly important in process chromatography and in laboratories having a high sample throughput. Analysis time

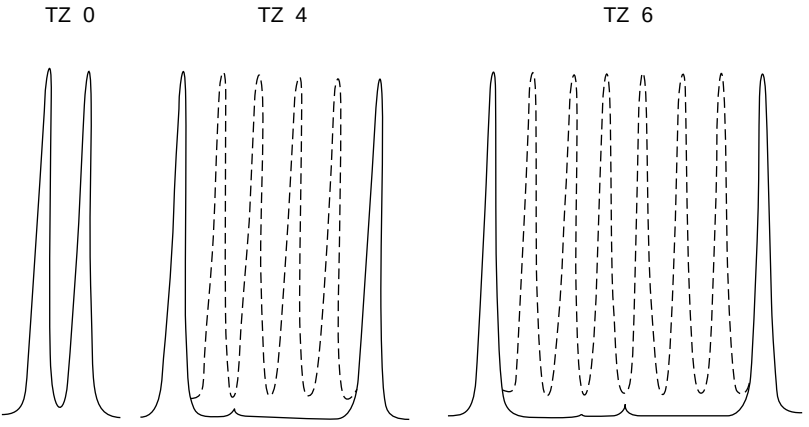


FIGURE 3.9 Illustration of separation number (Trennzahl).

is based on the solute component that is more readily sorbed. Using the equation for determination of retention time, we obtain

$$t = \frac{L(1+k)}{\overline{u}} = \frac{NH}{\overline{u}}(1+k) \tag{3.19}$$

and substituting the value for the required number of plates, n_{ne} *for* n (Equation 3.11), we arrive at an equation for the minimum analysis time t_{ne}:

$$t_{ne} = 16R_s^2 \frac{H}{\overline{u}} \left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1+k)^3}{(k)^2} \tag{3.20}$$

the term $H/\overline{u}$ can be expressed in terms of the modified van Deemter equation (Section 2.3.2, Equation 2.45).

$$\frac{H}{\overline{u}} = \frac{A}{\overline{u}} + \frac{B}{\overline{u}^2} + C_l + C_g \tag{3.21}$$

For minimum analysis time, high linear gas velocities are used; thus the first two terms on right side of Equation 3.22 may be neglected. Therefore,

$$\frac{H}{\overline{u}} = C_l + C_g \tag{3.22}$$

Substituting Equation 3.11 and 3.18 into Equation 3.18 we obtain

$$t_{ne} = n_{ne}(C_l + C_g)(1+k) \tag{3.23}$$

This equation indicates that minimal separation time depends on plate numbers, capacity factor, and resistance to mass transfer. It should be pointed out that the analysis times calculated from Equation 3.21 also depend on the desired resolution. Our example calculations were made on the basis of resolution $R_s = 1.5$. For a resolution of 1.00, even shorter analysis times can be achieved.

Figure 3.10 gives a representation of an idealized separation of component zones and the corresponding chromatographic peaks for a three-component system. With columns of increasing number of plates, we see better resolution as column efficiency increases.

3.6.5 Optimization of Packed-Column Separations

Examination of the parameters in the van Deemter expression (Equation 2.44), term by term, provides a basis for optimizing a packed column separation. The plate height, h, of a packed column may be represented as the sum of the eddy diffusion, molecular diffusion and mass transfer effects. Thus, to attain maximum column efficiency, each term in the plate height equation should be minimized:

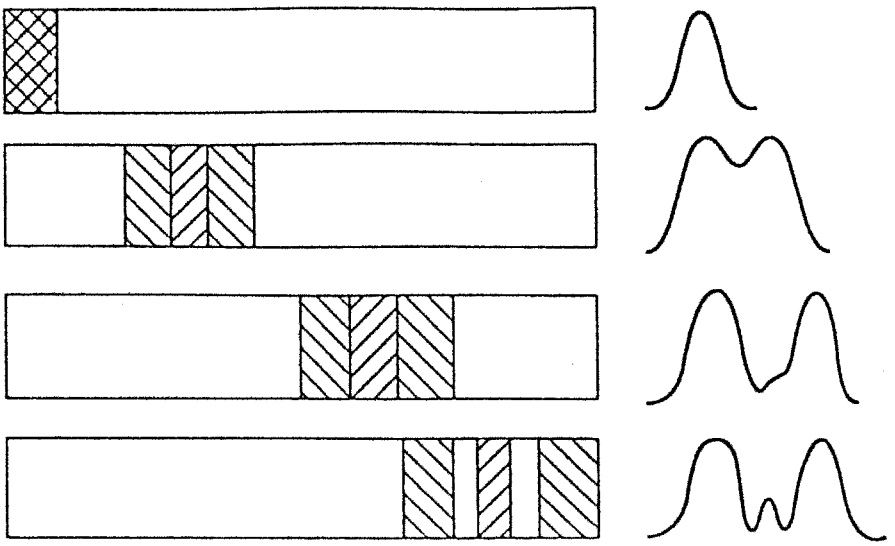


FIGURE 3.10 Idealized separation process with two major components and one minor component.

3.6.5.1 Eddy Diffusion

Also referred to as the *multiple-path effect*, eddy diffusion ($2\lambda d_p$) is minimized by using small particles of support materials. A support of 100/120 mesh produces a more efficient column than do 60/80-mesh particles and should be used whenever possible. A support of lower mesh, such as 80/100 or 60/80, should be selected to avoid a high pressure drop within a long column. This term is also independent of linear velocity or flowrate.

3.6.5.2 Molecular Diffusion

Molecular diffusion ($2\gamma D_g/\mu$) becomes significant at very low flowrates. This contribution may be minimized by using a carrier gas of high molecular weight (nitrogen, carbon dioxide, or argon) because their diffusion coefficient D_g is lower than that of a lower-molecular-weight carrier gas (helium or hydrogen) yielding a lower minimum in a H versus μ profile (Figure 3.11). Factors affecting the choice of helium versus hydrogen will be discussed later. However, other factors can override carrier-gas selection such as detector compatibility. Thus, helium is preferred over nitrogen as carrier gas with a thermal conductivity detector.

3.6.5.3 Mass Transfer Contribution

The mass transfer contribution $[8kd_f^2/\pi^2(1+k)^2 D_l]\mu$ requires compromises to be made. The magnitude of this term can be clearly minimized by decreasing film thickness (by using a packing having a lower loading of stationary phase). Therefore, column efficiency increases (and time of analysis decreases) with a decrease in stationary phase loading, as may been seen in Figure 3.4. If a

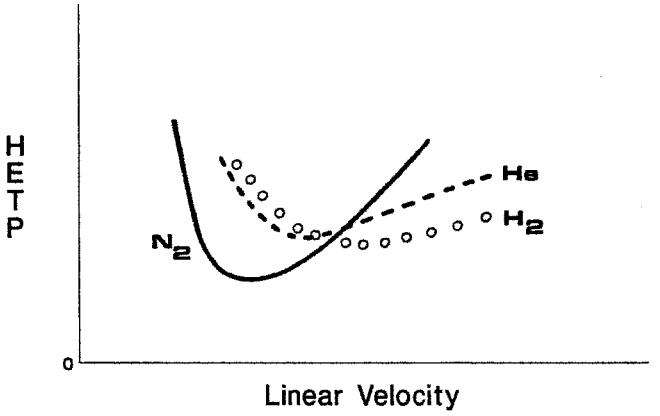


FIGURE 3.11 Plot of HETP versus linear velocity.

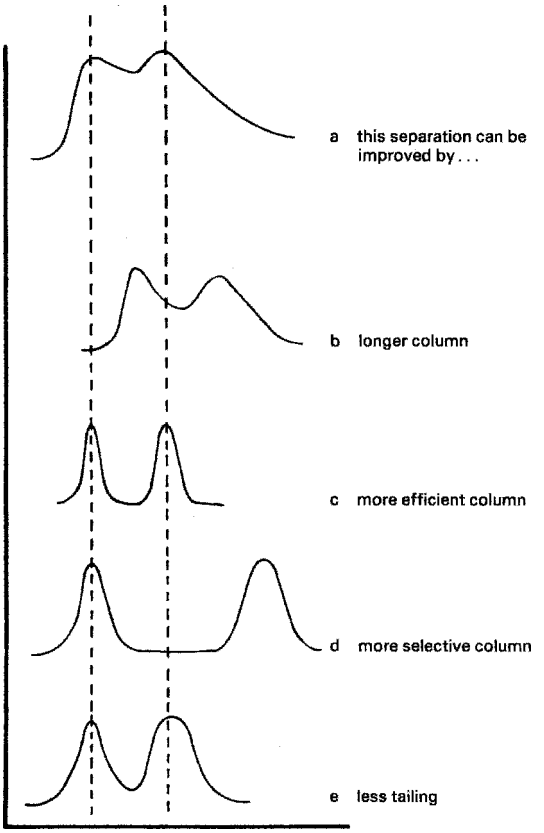


FIGURE 3.12 Effect of selected column changes on resolution. (Reproduced from Reference 20: W. A. Supina, in *Modern Practice of Gas Chromatography*, 2nd ed., R. L. Grob, ed., copyright 1985, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

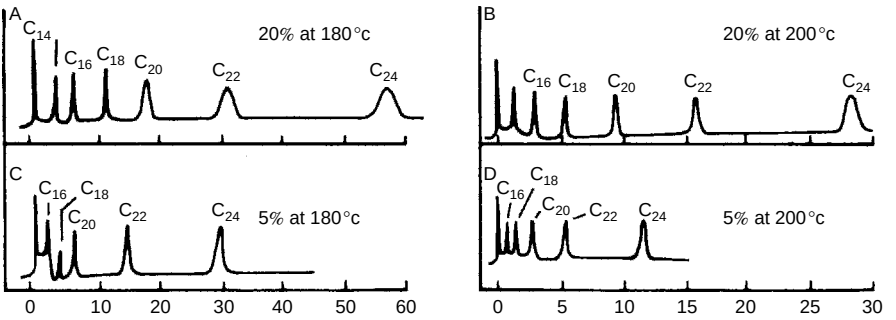


FIGURE 3.13 Effect of concentration of stationary phase and column temperature on sample resolution (methyl esters of fatty acids). (Reproduced from Reference 20: W. A. Supina, in *Modern Practice of Gas Chromatography*, 2nd ed., R. L. Grob, ed., copyright 1985, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)

support is too thinly coated, the exposure of active sites on the support may cause adsorption of solutes. A decrease in column temperature lowers the magnitude of the k' term; however, lowering of the column temperature also decreases D_l by increasing the viscosity of the stationary phase. Effects of the various changes in chromatographic parameters on resolution that can be implemented are schematically illustrated in Figure 3.12.

A relationship between stationary phase concentration and column temperature is depicted in Figure 3.13. Decreasing column temperature increases time of analysis; in order to have the same analysis time on a heavier loaded packing in an identical column at the same flowrate requires a higher column temperature.

3.7 COLUMN PREPARATION

Most laboratories today purchase columns of designated dimensions [length and inner and outer diameters (i.d., o.d.)] containing a specified packing such as untreated or treated support coated with a given liquid-phase loading, directly from a chromatography vendor. In-house preparation of packings and filling columns can be time-consuming and is false economy; more importantly, vendors can do the job better. Some supports are difficult to coat uniformly while some packings present a problem when filling a column. Nevertheless, guidelines and concise descriptions of recommended procedures to be employed for preparing a packed column are presented below.

3.7.1 Description of Coating Methods

The techniques of solvent evaporation and solution coating are most commonly used procedures for deposition of a liquid phase on a support. Solvent evaporation

is employed for coating supports with high concentrations (>15%) of viscous phases, while the solution-coating method produces a more uniform phase deposition and is more widely utilized.

In solvent evaporation, a known amount of stationary phase is dissolved in an appropriate solvent. A weighed amount of support is added to the solution and the solvent allowed to slowly evaporate from the slurry. Since all stationary phase is deposited on the support, stirring or thorough mixing is a necessity; otherwise nonuniform deposition of phase will result.

The technique of solution coating consists of the following steps:

1. A solution of known concentration of liquid phase in its recommended solvent is prepared.
2. A weighed amount of solid support is added to a known volume of this solution.
3. The resulting slurry is transferred into a Büchner funnel, with the remaining solvent removed by vacuum.
4. The volume of filtrate is measured.
5. After suction is completed, allow the wet packing to air-dry on a tray in a hood to remove residual solvent. Do not place damp packing into a laboratory drying oven!
6. The mass of liquid phase retained on the support is computed since the concentration of liquid phase in the solution is known.

This technique produces a uniform coating of a support, minimum generation of fines from the support particles, and minimum oxidation of the stationary phase. Further details about these procedures are described in References 12 and 20.

3.7.2 Tubing Materials and Dimensions

The nature and reactivity of the sample will govern the choice of tubing for packed-column GC. Of the available materials, glass is the most inert and the best material for most applications, although somewhat fragile. Overtightening a fitting attached to a glass column can cause the dreaded “ping” sound of broken glass. Utilization of a special torque wrench, which breaks apart itself rather than the glass column when a specific torque level is exceeded, is a good investment for a gas chromatographic laboratory. Empty glass columns need deactivation or silylation prior to packing. Usually this is accomplished by filling a thoroughly cleaned empty column with a 5% solution of DMDCS in toluene. After standing for 30 min, the column is rinsed successively with toluene and methanol, then purged with dry nitrogen, after which it is ready to be packed. Moreover, as opposed to a metal column, the use of glass permits direct visualization of how well a column is packed after filling and also after the column has been used for separations. Teflon tubing, also inert, is used for the analysis of sulfur gases, halogens, HF, HCl, but has a temperature limitation of 250°C.

Nickel tubing offers the attractive combination of the durability and strength of metal tubing with the favorable chemical inertness of glass. Stainless steel is the next least reactive material and is utilized for analysis of hydrocarbons, permanent gases, and solvents. As is the case with all metal tubing, stainless-steel columns should be rinsed with nonpolar and polar solvents to remove residual oil and greases. When used for the analysis of polar species, a higher grade of stainless-steel tubing with a polished inner surface is recommended. Copper and aluminum tubing have been employed for noncritical separations, but their use is not recommended and should be restricted to the plumbing of cylinder instrument gas lines. Oxide formation can occur on the inner surface of these materials, resulting in adsorptive tailing and/or catalytic problems under chromatographic conditions.

3.7.3 Glass Wool Plugs and Column Fittings

Chromatographic packings are retained within a column by a wad or plug of glass wool. Since the chemical nature of the wool closely resembles that of the glass column, it should also be deactivated by the same procedure used for glass columns. It is advisable to further soak the wool in a dilute solution of H_3PO_4 for the analysis of acidic analytes such as phenols and fatty acids. Untreated or improperly treated glass wool exhibits an active surface and can cause peak tailing. Alternatives to glass wool are stainless-steel frits and screens for gas chromatographic purposes, available from vendors of chromatographic supplies.

The ferrules and metal retaining nuts are used to form a leaktight seal of a column in a gas chromatograph. Criteria for selection of the proper ferrule material are column diameter, column tubing material, maximum column temperature, and whether the connection is designated for a single use or for multiple connections and disconnections. Ferrules fabricated from various materials for metal-to-metal, glass-to-metal, and glass-to-glass connections are commercially available for use with $\frac{1}{16}$-, $\frac{1}{8}$-, and $\frac{1}{4}$-in.-o.d. packed columns. The properties and characteristics of common types are presented in Table 3.12.

3.7.4 Filling the Column

The following procedure for packing columns, with practice, can produce the desired goal of a tight packing bed with minimum particle fracturing. First, a metal column is precoiled for easy attachment to the injector and detector of the instrument in which it is to be installed or a precoiled glass column configured for a specific instrument is procured from a vendor. Insert a large wad of glass wool partially into one end of the column, align the excess wool along the outside of the tubing, overlap the excess wool with vacuum tubing, and attach the other end of the vacuum tubing to a faucet aspirator or pump (12). After securing a small funnel to the other end of the column, add packing material in small incremental amounts into the funnel and gently tap the packing bed while applying suction. After the column is completely packed, insert a small piece of silanized glass wool into the inlet end of the column, disconnect the vacuum, and remove the

TABLE 3.12 Ferrule Materials for Packed Columns

Material	Temperature Limit (°C)	Properties
Metal		
Brass	250	Permanent connection on metal columns
Stainless steel	450	Permanent connection on metal columns
Teflon	250	Low upper temperature limit and cold-flow properties renders this material unsuitable for temperature programming and elevated temp. operation, reusable to some extent
Ceramic-filled	250	Isothermal use only; conforms easily to glass; used for connections to mass spectrometers
Graphite (G)	450	High temperature limit with no bleed or decomposition; soft and easily deformed upon compression; may be resealed only a limited number of times
Vespel 100% polyimide (PI)	350	Good reusability factor, can be used with glass, metal and Teflon columns; may seize on metal and glass columns with use at elevated temperature
Vespel/graphite 85% PI, 15% G	400	Excellent reusability; will not seize to glass or metal; performs better than graphite and Vespel alone; 60% PI composite seals with lesser torque and has added lubricity

vacuum tubing and the large wad of glass wool, replacing it with a smaller plug of silanized wool. This approach eliminates the exasperating sight of your packing material zipping out of the column during filling if a insufficiently tight wad of silanized glass wool was initially inserted into the outlet end of the column.

3.7.5 Conditioning the Column and Column Care

Before a column is used for analyses, it must be thermally conditioned by heating the column overnight at an oven temperature below the upper limit of the stationary phase with a normal flowrate of carrier gas. The column should not be connected to the detector during conditioning. The purpose of conditioning is the removal from the column residual volatiles and low-boiling species present in the stationary phase, which otherwise would produce an unsteady baseline at elevated column temperatures, commonly referred to as "column bleed," and contaminate the detector. Conditioning a column also helps in the redistribution of the liquid phase on the solid support. The degree of conditioning is dependent on the nature and amount of liquid phase in the column; usually heating a column overnight at an appropriate elevated temperature produces a steady baseline under chromatographic conditions the following day. Analyses using the more sensitive detectors (ECD, NPD, MS) may require an even longer column conditioning period.

The following guidelines can prolong the lifetime of a column:

1. Any gas chromatographic column, new or conditioned, packed or capillary, should be purged with dry carrier gas for 15–30 min before heating to a final elevated temperature to remove the detrimental presence of air.
2. A column should not be rapidly or ballistically heated to an elevated temperature but should be heated by slow to moderate temperature programming to the desired final temperature.
3. Excessively high conditioning and operating temperatures reduce the lifetime of any gas chromatographic column.
4. Use "dry" carrier gas or install a moisture trap in the carrier-gas line. Do not inject aqueous sample on a column containing a stationary phase intolerant of water.
5. The accumulation of high-boiling compounds from repetitive sample injections occurs at the inlet end of the column and results in discoloration of the packing. It is a simple matter to remove the discolored segment of packing and replace it with fresh packing material. This action prolongs the column lifetime.
6. Do not thermally shock a column by disconnecting it while it is hot. Allow the column to cool to ambient temperature prior to disconnection. Packings are susceptible to oxidation when hot.
7. Cap the ends of a column for storage to prevent air and dust particles from entering the column. Save the box in which a glass column was shipped for safe storage of the column.

PART 3 CAPILLARY COLUMN GAS CHROMATOGRAPHY

3.8 INTRODUCTION

The capillary column, also referred to as an *open tubular column* because of its open flow path, offers a number of advantages over the packed column. These merits include vastly improved separations with higher resolution, reduced time of analysis, smaller sample size requirements, and often higher sensitivities. The arrival of fused silica as a capillary-column material had a major impact on capillary gas chromatography and, in fact, markedly changed the practice of gas chromatography. In 1979 the number of sales of gas chromatographs with capillary capability was less than 10%; this figure increased to 60% by 1989 (42) and only will continue to increase as further developments in capillary-column technology and instrumentation are made. Here in Part 3, theoretical and practical considerations of the capillary column are discussed.

3.8.1 Significance and Impact of Capillary GC

Marcel Golay is credited with the discovery of the capillary column. In 1957, Golay presented the first theoretical treatment of capillary-column performance

when he illustrated that a long length of capillary tubing having a thin coating of stationary-phase coating the narrow inner diameter of the tube offered a tremendous improvement in resolving power compared to a conventional packed column (43). Such a column is also often referred to as a wall-coated open tubular column (WCOT). The high permeability or low resistance to carrier-gas flow of capillary column enables a very lengthy column to generate a large number of theoretical plates.

In contemporary practice, separations of high resolution are attainable by capillary GC, as illustrated in the chromatogram in Figure 3.14, which was generated with a conventional fused-silica capillary column. An exploded view of this chromatogram of a gasoline-contaminated jet A fuel mixture with a data acquisition system indicates the presence of over 525 chromatographic peaks. Because of its separation power, capillary gas chromatography has become synonymous with the term "high-resolution gas chromatography."

3.8.2 Chronology of Achievements in Capillary GC

The first column materials employed in the developmental stage of the technique were fabricated from plastic materials (Tygon and nylon) and metal (aluminum, nickel, copper, stainless steel, and gold). Plastic capillaries, which are thermoplastic in nature, had temperature limitations, whereas metallic capillary columns had the disadvantage of catalytic activity. Rugged, flexible stainless-steel columns rapidly became state-of-the-art, and were widely used for many applications, mainly for petroleum analyses. The reactive metallic surface proved to be unfavorable in the analysis of polar and catalytically sensitive species. In addition,

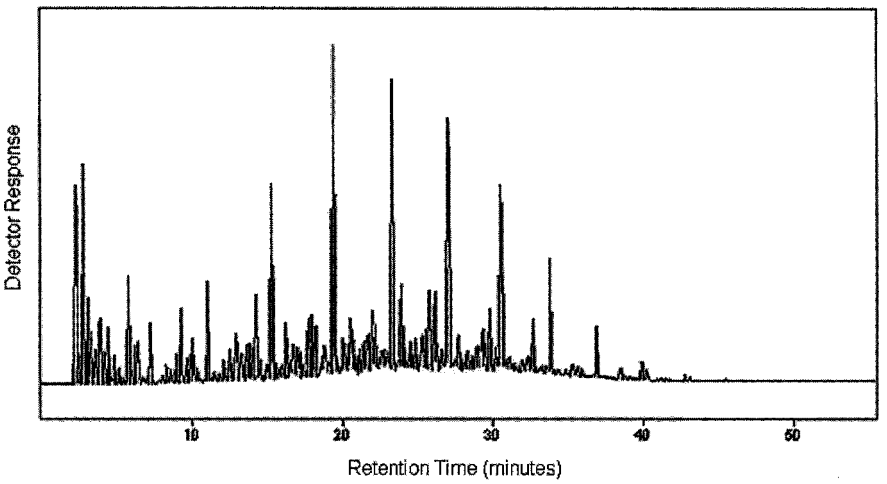


FIGURE 3.14 Chromatogram of a sample of jet A fuel contaminated with gasoline on 30-m × 0.25-mm-i.d. HP-1 (0.25-μm film). Column temperature conditions: 30°C (5 min), 2°C/min to 250°C; split injection (100–1). Det: FID.

only the split-injection mode was available for quite some time for the introduction of the small quantities of sample dictated by a thin film of stationary phase within the column. As the surface chemistry of glass was gradually studied and understood, capillaries made of borosilicate and sodalime glass became popular in the 1970s and replaced metal capillary columns (44). Here the metal oxide content and presence of silanol groups on the glass surface necessitated carefully controlled deactivation and coating procedures, but separations obtained on glass capillaries were clearly superior to those obtained with metal capillaries. The fragility of glass often proved to be problematic, requiring restraightening of a capillary end upon breakage with a minitorch followed by a recoating of the straightened portion with a solution of stationary phase to deactivate the straightened segment. Patience was also helpful! Today equivalent or superior separations with a fused-silica capillary column can be generated with the additional feature of ease of use.

The most significant advancement in capillary gas chromatography occurred in 1979 when Dandeneau and Zerenner of Hewlett–Packard (at the time) introduced fused silica as a column material (45,46). The subsequent emergence of fused silica as the column material of choice for high-resolution gas chromatography is responsible for the widespread use of the technique and has greatly extended the range of gas chromatography. In the next two decades, there were several other major developments in capillary gas chromatography. Instrument manufacturers responded to the impact of fused-silica columns by designing chromatographs with injection and detector systems optimized in performance for fused-silica columns. There were also concurrent advances in the area of microprocessors. Reporting integrators and fast data acquisition systems with increased sampling rates now are available to be compatible with the narrow bandwidths of capillary peaks. The stature of the capillary column has been further enhanced by continuing improvements in the performance and thermal stability of the stationary phase within the column. A column containing a crosslinked phase, a silarylene phase, or silphenylene phase, for example, has an extended lifetime because it has high thermal stability and can tolerate large injection aliquots of solution without redistribution of the stationary phase. Inlet discrimination was addressed with the development of on-column injection, the programmed temperature vaporizer, electronic pressure-controlled injection and, more recently, the large volume injector with cool on-column inlet mode.

Since Golay's proposal of the use of the capillary column, capillary gas chromatography has exhibited spectacular growth, maturing into a powerful analytical technique. Some of the more notable achievements in capillary gas chromatography are listed in Table 3.13.

3.8.3 Comparison between Packed and Capillary Columns

Three stages in the evolution of the capillary-column technology are presented in Figure 3.15: a packed-column separation and two separations with a stainless-steel and glass capillary column. Better resolution is evident with the capillary

TABLE 3.13 Advancements in Capillary Gas Chromatography

Year	Achievement
1958	Theory of capillary column performance, GC Symposium in Amsterdam
1959	Sample inlet splitter
1959	Patent on capillary columns by Perkin–Elmer
1960	Glass drawing machine developed by Desty
1965	Efficient glass capillary columns
1975	First capillary column symposium
1978	Splitless injection
1979	Cold on-column injection
1979	Fused silica introduced by Hewlett–Packard
1981–1984	Deactivation procedures and crosslinked stationary phases
1983	Megabore column introduced as an alternative to the packed column
1981–1988	Interfacing capillary columns with spectroscopic detectors (MS, FTIR, AED)
1992–2002	Programmed temperature vaporizer electronic pressure- controlled sample inlet systems; MS-grade columns, solid-phase microextraction sampling techniques, large-volume injectors, advances in GCMS, more affordable bench–top GCMS systems

Source: Some data here courtesy of Agilent Technologies.

chromatograms because more peaks are separated and smaller peaks can be detected. The superior performance of the glass capillary column is clearly apparent.

In addition to providing a separation where peaks have narrower bandwidths compared to a packed-column counterpart, a properly prepared fused-silica capillary column, which has an inert surface (less potential for adverse adsorptive effects toward polar species), yields better peak shapes; bands are sharper with less peak tailing, which facilitates trace analysis as well as provides more reliable quantitative and qualitative analyses. Sharp, narrow bands of the trace components present in a capillary chromatogram such as that in Figure 3.14 have increased peak height relative to the peak of the same component at identical concentration in a packed-column chromatogram where the peak may be unresolved or disappear in the baseline noise. Moreover, because of the low carrier-gas flowrate, greater detector sensitivity, stability, and signal-to-noise levels are possible with a capillary column. One drawback of the capillary column, though, is its limited sample capacity, which requires dedicated inlet systems to introduce small quantities of sample commensurate with a low amount of stationary phase. Operational parameters of packed and capillary columns are further contrasted in Table 3.14.

The superior performance of a capillary column can be further viewed in the following manner. Because of the geometry and flow of a gas through a packed bed, molecules of the same solute can take a variety of paths through the column

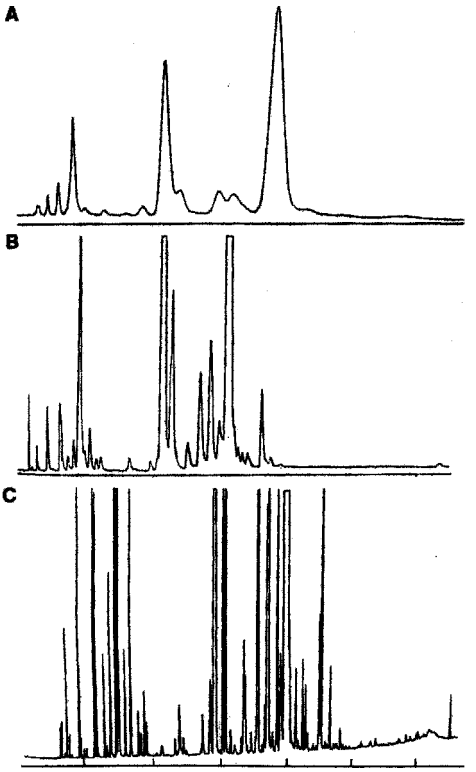


FIGURE 3.15 Optimized separations of peppermint oil on (a) 6-ft × 0.25-in.-i.d. packed column, (b) 500-ft × 0.03-in.-i.d. stainless-steel capillary column; and (c) 50-m × 0.25-mm-i.d. glass capillary column. Stationary phase on each column was Carbowax 20M. [W. Jennings, *J. Chromatogr. Sci.* **17**, 637 (1977). Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a Division of Preston Industries, Inc.]

TABLE 3.14 Comparison of Wall-Coated Capillary Columns With Packed Columns

	Packed	Capillary
Length (m)	1–5	5–60
Inner diameter (mm)	2–4	0.10–0.53
Plates per meter	1000	5000
Total plates	5000	300,000
Resolution	Low	High
Flowrate (mL/min)	10–60	0.5–15
Permeability (10^7 cm^2)	1–10	10–1000
Capacity	10 μg/peak	>100 ng/peak
Liquid film thickness	10	0.1–1 (μm)

Source: Data obtained in References 47 and 48.

enroute to the detector (via eddy diffusion), whereas in a capillary column all flow paths have nearly equal length. The open geometry of a capillary column causes a lower pressure drop, allowing longer columns to be used. Since a packed column contains much more stationary phase, often thickly coated on an inert solid support, there are locations in the packing matrix where the stationary phase spans or spreads over to adjacent particles (Figure 3.2a). Some molecules of the same component encounter thinner regions of stationary phase, whereas other molecules have increased residence times in these thicker pools of phase, all of which create band broadening. On the other hand, a capillary column contains a relatively thin film of stationary uniformly coated on the inner wall of the tubing. These factors, collectively considered, are responsible for the sharp band definition and narrow retention time distribution of molecules of a component eluting from a capillary column.

At higher column oven temperatures with increased linear velocity of carrier gas, capillary separations can be achieved that mimic those on a packed column but with a shortened time of analysis. The reduced amount of stationary phase in a capillary column imparts another advantage to the chromatographer, namely, one observes less bleed of stationary phase from the column at elevated temperatures and this means less detector contamination. Theoretical considerations of the capillary column are discussed in Section 3.10.

3.9 CAPILLARY COLUMN TECHNOLOGY

3.9.1 Capillary Column Materials

After Desty developed a glass drawing and coiling apparatus (49), focus shifted away from metal capillary columns to fabrication of columns from more inert borosilicate and sodalime glass. Glass is inexpensive and readily available, and glass columns could be conveniently drawn in house with dimensions (length and inner diameter) tailored to individual needs. Investigators quickly realized that this increase in the column inertness of glass was at the expense in flexibility. With fused silica, a column could be fabricated from a material having the flexibility of stainless steel with an inner surface texture more inert than glass. Thus, fused silica quickly replaced glass as the capillary-column material of choice.

3.9.1.1 Fused-Silica and Other Glasses

To cultivate an understanding for the widespread use of fused silica as a column material for capillary GC, it is helpful to examine the chemical structures of glasses in Figure 3.16, which have been used as column materials and the corresponding metal oxide concentration data presented in Table 3.15. Column activity may be attributed to exposed silanol groups and metal ions on the surface of a glass capillary. While sodalime borosilicate glasses, for example, have percentage levels of metal oxides, the metal content of synthetic fused silica is less than 1 ppm. Although quartz tubing is commercially available, its metal oxide content (10–100 ppm) is considered to be too great for use in capillary

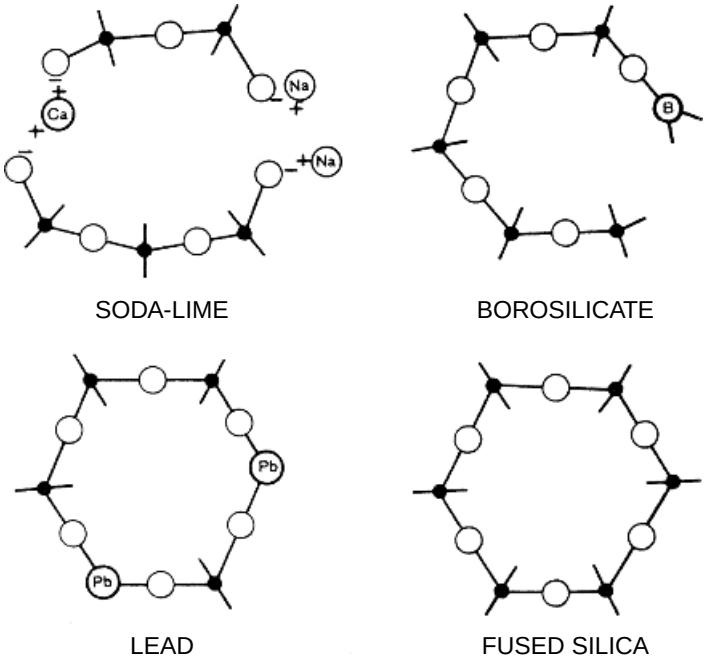


FIGURE 3.16 Schematic representations of the structures of different glasses (reproduced from Reference 51 and reprinted with permission of Dr. Alfred Huethig Publishers).

TABLE 3.15 Approximate Glass Composition (%)

Glass	SiO_2	Al_2O_3	Na_2O	K_2O	CaO	MgO	B_2O_3	PbO	BaO
Sodalime (Kimble R6)	68	3	15	—	6	4	2	—	2
Borosilicate (Pyrex 7740)	81	2	4	—	—	—	13	—	—
Potash soda lead (Corning 120)	56	2	4	9	—	—	—	29	—
Fused silica	100	—	—	—	—	—	—	—	—

Less than 1 ppm total metals.

Source: Data abstracted from References 47 and 51.

gas chromatography (50). Metal oxides are considered to be Lewis acids and can serve as adsorptive sites for electron–donor species such as ketones and amines and as an active site for species with *p*-bonding capability (aromatics and olefins). Boron impurities in glass also act as Lewis acid sites capable of chemisorbing electron donors (51). The absence of these adsorptive sites in fused silica is responsible for its remarkable inertness and is a direct result of the

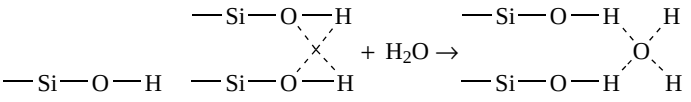
synthesis of this material. However, the hydroxyl groups attached to tetravalent silicon atoms are of paramount significance because they can contribute residual column activity.

Synthetic fused silica is formed by introducing pure silicon tetrachloride into a high-temperature flame followed by reaction with the water vapor generated in the combustion (51,52). The process can be described by the reaction

$$SiCl_4 + 2H_2O \rightarrow SiO_2 + 4HCl \tag{3.24}$$

There are three distinct categories of silanol groups present on the surface of fused silica shown in Figure 3.17:

1. There are free silanol groups, which are acidic adsorptive sites with $K_a = 1.6 \times 10^{-7}$. The surface concentration of free silanols on fused silica has been calculated to be 6.2 μmol/m^2. This type of silanol group has a direct bearing on column behavior.
2. Geminal silanols, in which two hydroxyl groups are attached to the same silicon atom, are also present at a concentration of 1.6 μmol/m^2 (53).
3. There are vicinal silanol functionalities characterized by hydroxyl groups attached to adjacent silicon atoms. Here steric effects become important. For instance, vicinal silanols represent a rather weak adsorptive site but in the presence of water can be rendered active (51):



Adsorption site: Strong Weak or none Strong

(3.25)

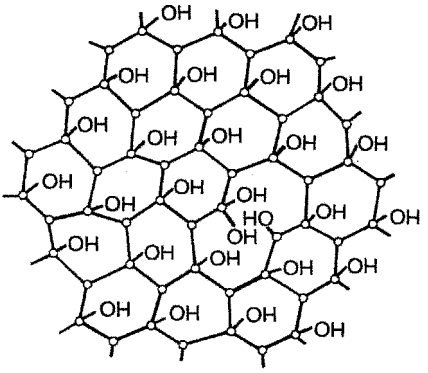


FIGURE 3.17 Probable structure of fused silica (reproduced from Reference 51 and reprinted with permission of Dr. Alfred Huethig Publishers).

If the interatomic distance of neighboring oxygen atoms is between 2.4 and 2.8 Å, the groups are hydrogen-bonded; if this distance exceeds 3.1 Å, hydrogen bonding does not occur and the free silanol behavior dominates (54). Bound silanol groups can dehydrate producing siloxane bridges under certain conditions. More detailed information on the complexities of silica surface chemistry may be found in the books by Jennings (51,52) and Lee et al. (47).

3.9.1.2 Extrusion of a Fused-Silica Capillary Column

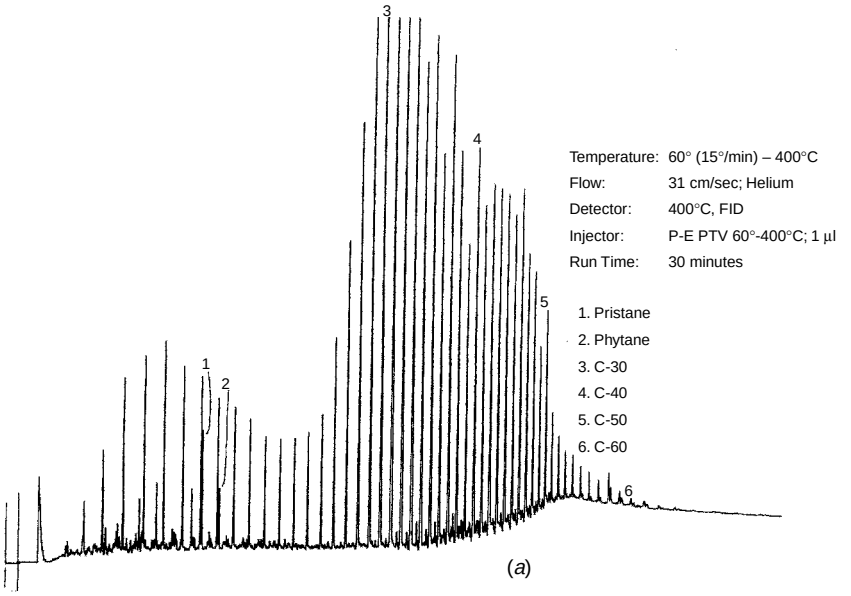
Three steps are involved in the preparation of a fused silica column: (1) the high-temperature extrusion of the blank capillary tubing from a preform, where the capillary receives a protective outer coating in the same process; (2) the deactivation of the inner surface of the column; and (3) the uniform deposition of a stationary phase of a desired film thickness on the deactivated inner surface. In this section the extrusion of fused silica will be described; the procedures employed for the deactivation and coating of fused-silica capillaries are presented in the following section.

Prior to extrusion the fused silica preform is usually treated with dilute hydrofluoric acid to remove any imperfections and deformations present on the inner and outer surfaces and then rinsed with distilled water and followed by annealing (55). In a cleanroom atmosphere, the preform is vertically drawn through a furnace maintained at approximately 2000°C. Guidance and careful control of the drawing process is achieved by focusing an infrared laser beam down the middle of the capillary in conjunction with feedback control electronic circuitry in order to maintain uniformity in the specifications of the inner and outer diameters in the final product.

Fused silica drawn in this manner exhibits a very high tensile strength and has excellent flexibility due to the thin wall of the capillary. However, the thin wall of the capillary is subject to corrosion on exposure to atmospheric conditions and is extremely fragile. To eliminate degradation and increase its durability, the fused-silica tubing receives a protective outer coating, usually of polyimide, although other coating materials have been used, including silicones, gold, vitreous carbon, and polyamides. Polyimide, which also serves as a water barrier, is most widely used because it offers temperature stability to 400°C. The color of polyimide seems to vary slightly from one column manufacturer to another, with no effect, however, on column performance. An excellent historical review on the story behind the technology and extrusion of fused-silica tubing was published in 2002 (56). Jennings prepared a fascinating recounting of his own personal perspectives on the development and commercialization of the capillary column (57).

3.9.1.3 Aluminum-Clad Fused-Silica Capillary Columns

There are number of application areas requiring columns to be operated at or above 400°C, such as the analysis of waxes, crude oils, and triglycerides. These have driven efforts to replace the polyimide outer coating with a thin (20-mm) layer of aluminum and extend the temperature range of capillary gas chromatography, as illustrated in the chromatograms of high-temperature capillary separations



TRIGLYCERIDE LEGEND	
m = Myristic Acid, C-14:0	o = Oleic Acid, C-18:1
p = Palmitic Acid, C-16:0	l = Linoleic Acid, C-18:2
s = Stearic Acid, C-18:0	s = Eicosanoic Acid, C-20:0

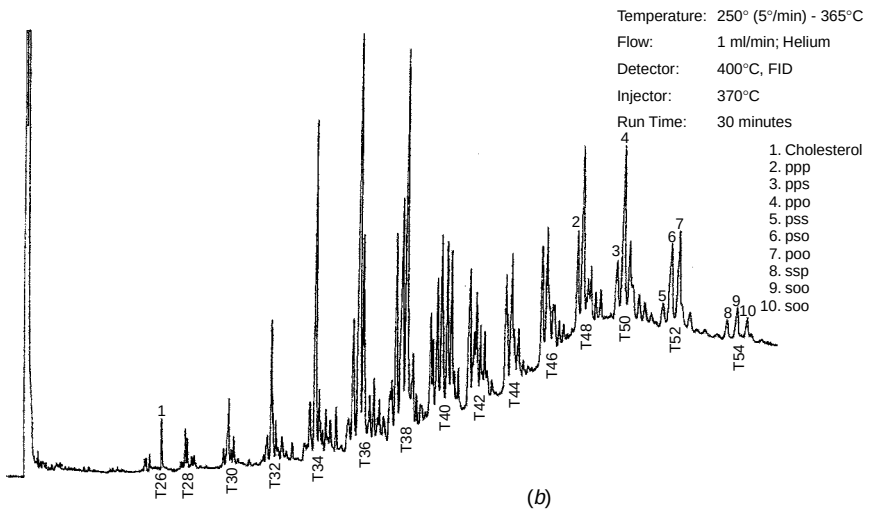
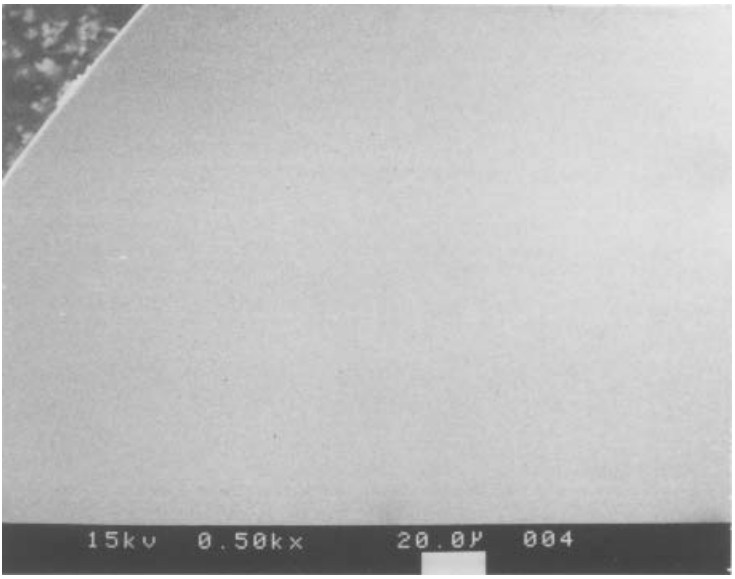


FIGURE 3.18 Chromatogram of separation of (a) Canadian wax on 15-m × 0.25-mm-i.d. aluminum-clad capillary column (0.1-µm film) and (b) triglycerides on a 25-m × 0.25-mm-i.d. aluminum-clad capillary column (0.1-µm film) (chromatograms courtesy of the Quadrex Corp.)

in Figure 3.18. An aluminum-clad capillary column has excellent heat transfer while maintaining the same flexibility and inertness of the fused-silica surface as the polyimide-coated columns. Trestianu and Gilioli showed that for an alkane of high carbon number the elution temperature on a high-temperature column is 100°C lower than with a corresponding packed column (58). It must be emphasized here that to obtain optimum column performance, the injection mode is critical. Cold on-column or programmed temperature vaporizer injectors are recommended to avoid inlet discrimination problems for the analysis of solutes of high molecular weight with this type of capillary column.

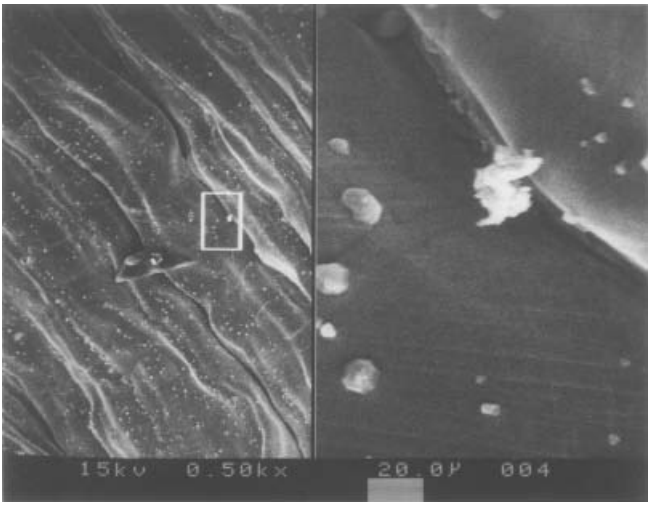
3.9.1.4 Fused-Silica-Lined Stainless-Steel Capillary Columns

A third type of protective outer coating, stainless steel, for fused silica offers an alternative to aluminum-clad fused silica for elevated column temperatures. This technology is the inverse of that for polyimide-clad fused-silica capillary where a layer of fused silica is deposited on the inner surface of a stainless capillary. In Figure 3.19 scanning electron micrographs are displayed to compare the rough surface of stainless steel with the smooth surface of untreated fused silica and the surface of stainless steel after a micron meter layer of deactivated fused silica

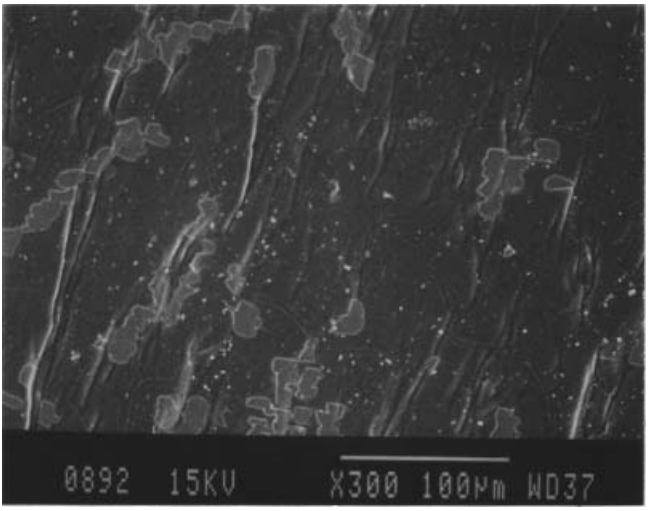


(*a*)

FIGURE 3.19 Scanning electron micrographs of (a) untreated fused silica, (b) rough inner surface of stainless-steel capillary tubing, and (c) the smoother inner surface of the stainless-steel capillary tubing after deposition of a thin layer of fused silica; part (c) also illustrates regions where fused-silica lining was selectively removed to expose untreated stainless-steel surface below (scanning electron micrographs courtesy of the Restek Corp.)



(*b*)



(*c*)

FIGURE 3.19 (*Continued*)

is bonded to its interior wall, termed *silcosteel*. In addition to high thermal stability, a distinguishing feature of a fused-silica-lined, thin-walled stainless-steel capillary column is that it can be coiled in a diameter less than 4 in. (compared to larger diameters with polyimide-clad fused silica) without breakage, making it a very favorable column material for process control and portable gas chromatographs where size of a the column oven, shock resistance, and ruggedness

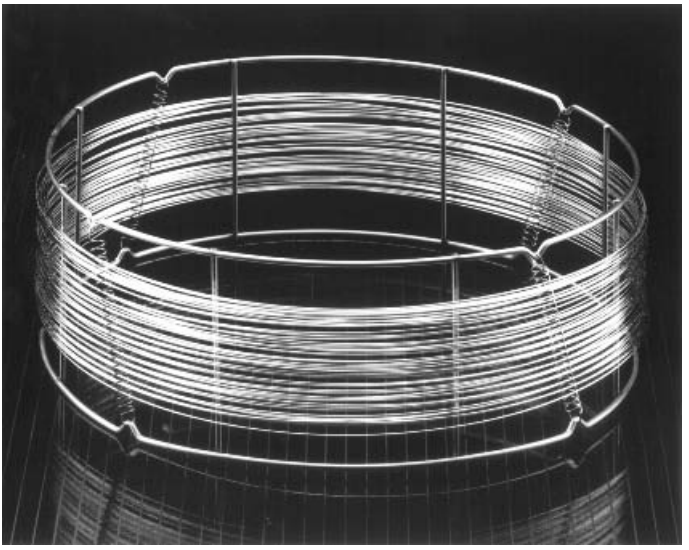
become limiting factors. Figure 3.20 shows photographs of aluminum-clad and fused-silica-lined stainless-steel capillaries. Along the same line, an aluminum capillary lined with quartz that was coated with carbon black has been evaluated for the analysis of amines, volatile organic compounds (VOCs), and oil products (59).

3.9.2 Preparation of a Fused-Silica Capillary Column

Most users of a modern capillary column regard it as a high-precision and sophisticated device and purchase columns from a vendor. Few give any thought to the steps involved in column preparation. Their number one priority is understandably the end result of accurate and reproducible chromatographic data that the column can provide. In this section, an overview of deactivation and coating of a fused-silica column with stationary phase is discussed.

3.9.2.1 Silanol Deactivation

For maximum column performance, blank or raw fused-silica tubing must receive pretreatment prior to the final coating with stationary phase. The purpose of pretreatment is twofold: to cover up or deactivate active surface sites and to create a surface more wettable by the phase. The details of the procedure are dependent on the stationary phase to be subsequently coated, but deactivation



(*a*)

FIGURE 3.20 Photographs of metal-clad capillary columns: (a) aluminum-clad capillary column (photograph courtesy of the Quadrex Corp.); (b) fused-silica-lined stainless-steel capillary column (lower) and polyimide-clad fused-silica capillary columns (upper); photographs courtesy of the Restek Corporation.

(*b*)

FIGURE 3.20 (*Continued*)

is essential for producing a column having a uniform film deposition along the inner wall of the capillary.

Although metal ions are not a factor with fused silica, the presence of silanol groups still must be addressed; otherwise the column has residual surface activity. Column activity can be demonstrated in several ways. The chromatographic peak of a given solute can completely disappear, partially disappear as its size diminishes, or exhibit tailing. A chromatogram of a test mixture showing the activity of an uncoated fused-silica column is displayed in Figure 3.21a; the inherent acidity associated with surface silanol groups is responsible for the complete disappearance of the basic probe solute, 2,6-dimethylaniline. When this column is deactivated with a precoating of Carbowax 20M, the residual surface column is considerably reduced (Figure 3.21b).

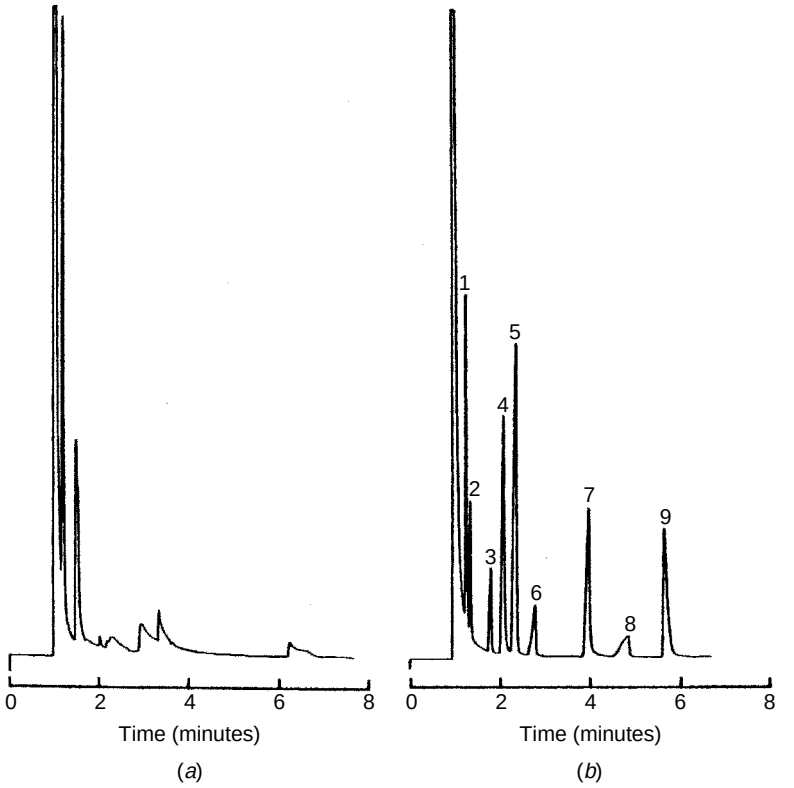


FIGURE 3.21 Chromatograms of an activity mixture on 15-m × 0.25-mm (a) uncoated fused silica and (b) fused-silica capillary column after deactivation with Carbowax 20M. Column temperature: 70°C; 25 cm/s He; split injection (100–1). Peaks: (1) *n*-dodecane, (2) *n*-tridecane, (3) 5-nonanone, (4) *n*-tetradecane, (5) *n*-pentadecane, (6) 1-octanol, (7) naphthalene, (8) 2,6-dimethylaniline, and (9) 2,6-dimethylphenol. (Reference 7.)

A variety of agents and procedures have been explored for deactivation purposes (60–74). For subsequent coating with nonpolar and moderately polar stationary phases such as polysiloxanes, fused silica has been deactivated by silylation at elevated temperatures, thermal degradation of polysiloxanes and polyethylene glycols, and the dehydrocondensation of silicon hydride polysiloxanes (71,75–79).

Blomberg has published a comprehensive review of deactivating methods using polysiloxanes (80). One approach has been suggested by Schomburg et al. (77), who prepared columns having excellent thermal stability with polysiloxane liquid phases as deactivators and proposed that the decomposition products formed at the elevated temperatures chemically bond to surface silanols. Surface stationary-phase compatibility has also been achieved with cyclic siloxanes having the same side functional groups as the silicone stationary phase.

Octamethylcyclotetrasiloxane (D_4) has been decomposed at 400°C by Stark et al. (81), who postulated that the process involved opening the D_4 ring to form a 1,4-hydroxyoctamethyltetrasiloxane. They indicate that a terminal hydroxyl group interacts with a protruding silanol group eliminating water, and in a secondary reaction the other hydroxyl reacts with another silanol or even a tetrasiloxane. Well-deactivated capillary columns can be prepared by this technique (82). In Figure 3.22 the effectiveness of the D_4 deactivation procedure is demonstrated for both acidic and basic test mixtures where the components have excellent band profiles. Woolley et al. outlined an easily implemented deactivation procedure employing the thermal degradation of polyhydrosiloxane at about 260°C, where the silyl hydride groups undergo reaction with surface silanols to form rather stable Si–O–Si bonds and also hydrogen gas (78). This method has the merits of a reaction time less than a hour, a relatively low reaction temperature, and a high degree of reproducibility. A representation of selected deactivated surface textures is displayed in Figure 3.23.

Carbowax 20M has also been successfully used to deactivate column surfaces (84,85). After coating a thin film of Carbowax 20M, for example, on the column wall, the column is heated to 280°C, then exhaustively extracted with

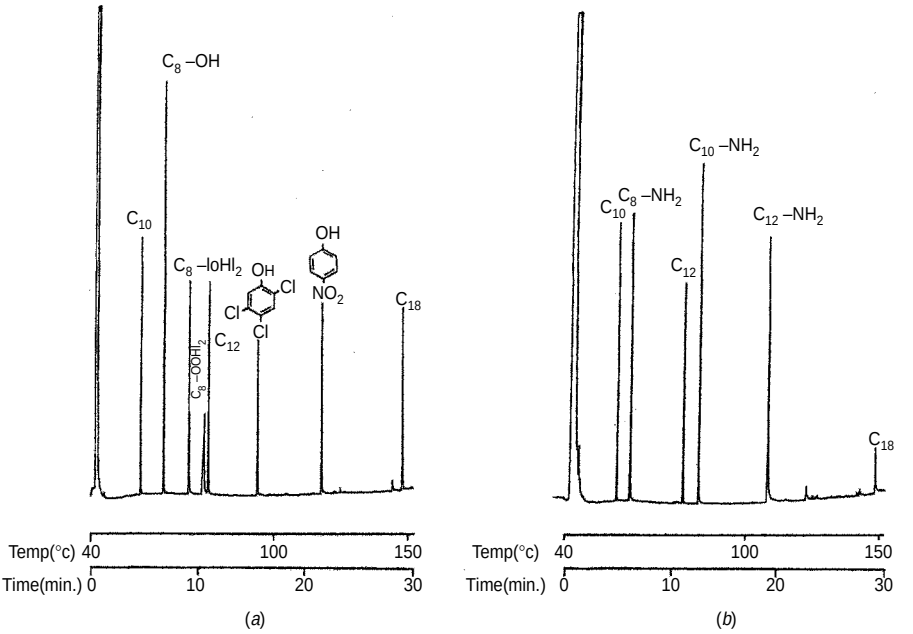


FIGURE 3.22 Chromatogram illustrating the inertness attainable on a D_4-deactivated SE-54 (0.25-μm) fused-silica column with (a) an acidic test mixture and (b) a basic test mixture; temperature programmed from 40°C at 4°C/min after 2 min isothermal hold; H_2 carrier gas at 45 cm/s (reproduced from Reference 82 and reprinted with permission of Elsevier Science Publishers).

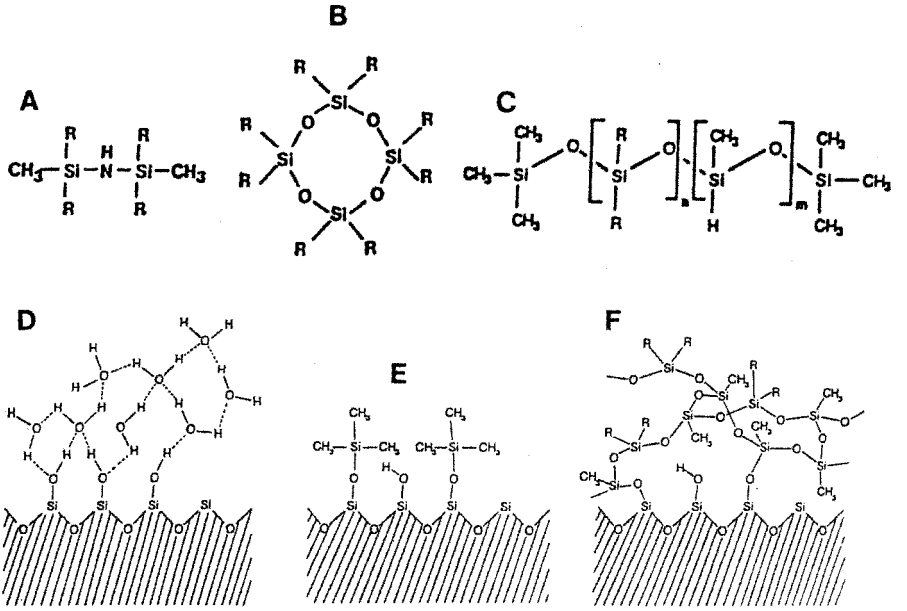


FIGURE 3.23 Selected reagents used for deactivation of silanol groups: (a) disilazanes, (b) cyclic siloxanes, (c) silicon hydride polysiloxanes. Lower portion is a view of fused-silica surface with (d) adsorbed water (e) after deactivation with a trimethylsilylating reagent and (f) after treatment with a silicon hydride polysiloxane. (Reproduced from Reference 83 and reprinted with permission from Elsevier Science Publishers.)

solvent, leaving a nonextractable film of Carbowax 20M on the surface. Both apolar and polar stationary phases, including Carbowax, can then be coated on capillaries subjected to this pretreatment (86). Dandeneau and Zerenner used this procedure to deactivate their first fused-silica columns (45). Other polyethylene glycols used for deactivating purposes have been Carbowax 400 (87), Carbowax 1000 (88), and Superox-4 (89). Moreover, when a polar polymer is used for deactivation, it may alter the polarity of the stationary phase, and this effect becomes particularly problematic with a thin film of a nonpolar phase where the resulting phase has retentions of a mixed phase. Furthermore, silazanes and cyclic silazanes, as deactivating agents, ultimately yield a basic final column texture, whereas chlorosilanes, alkoxysilanes hydrosilanes, hydrosiloxanes, siloxanes, and Carbowax produce an acidic column (4). In essence, a deactivation procedure imparts different residual surface characteristics and is often selected with the stationary phase as well as the application in mind. Many column manufacturers offer base-deactivated columns (and base-deactivated inlet liners) with several stationary phases for successful chromatography of amines. There have been two additional approaches to deactivation: (1) the coating of a layer of polypyrrone on the inner surface of the tubing prior to the deposition of the stationary phase, thereby circumventing the temperature limitation

of polyimide in high temperature applications (90); and (2) the "Siltek" process, where deactivation is achieved via a vapor deposition process (91,92) as opposed to procedures using agents such as liquid silazane or chlorosilane. An alternative procedure is the utilization of OH-terminated stationary phases where deactivation and immobilization of the phase occurs in a single-step process (Section 3.11.5).

3.9.2.2 Static Coating of Capillary Columns

The goal in coating a capillary column is the uniform deposition of a thin film, ranging from 0.1 to 8 μm in thickness, on the inner wall of a length of clean, deactivated fused-silica tubing. Jennings (94) has reviewed the various methods for coating stationary phases. The static method of coating is discussed here because it is most widely used today by column manufacturers.

This procedure was first described by Bouche and Verzele (95), who initially completely filled the column with a solution of known concentration of stationary phase. In this procedure, one end of the column is sealed and the other is attached to a vacuum source. As the solvent evaporates, a uniform film is deposited on the column wall. The column must be maintained at constant temperature for uniform film deposition. The coating solution should be free of microparticulates and dust, be degassed so no bumping occurs during solvent evaporation, and there should be no bubbles in the column. Pentane is the recommended solvent because of its high volatility and should be used wherever stationary phase solubility permits. Evaporation time is approximately half that required to evaporate methylene chloride. The static coating technique offers the advantage of an accurate determination of the phase ratio (Section 3.10.3) from which the film thickness of the stationary phase can be calculated.

3.9.2.3 Capillary Cages

Since the ends of flexible fused-silica capillary tubing are inherently straight, columns must be coiled and confined on a circular frame, also called a "cage" (Figure 3.20). The capillary column can then be mounted securely in the column oven of a gas chromatograph. Fused-silica capillary columns of 0.10–0.32 mm i.d. are wound around a 5- or 7-in.-diameter cage whereas an 8-in. cage is used with megabore columns (0.53 mm i.d.). Installation of a capillary column is greatly facilitated, since the ends of a fused-silica column can easily be inserted at the appropriate recommended lengths into sample inlets and detector systems. The ultimate in gas chromatographic system inertness is attainable with on-column injection, where a sample encounters only fused silica from the point of injection to the tip of a FID flame jet.

3.9.2.4 Test Mixtures for Monitoring Column Performance

The performance of a capillary column can be evaluated with a test mixture whose components and resulting peak shapes serve as monitors of column efficiency and diagnostic probes for adverse adsorptive effects and the acid/base character of a column. These mixtures are used by column manufactures in the quality control of their columns and are likewise recommended for the chromatographic laboratory.

A chromatogram of a test mix and a report are usually supplied with a commercially prepared column. Using the same indicated chromatographic conditions, the separation should be duplicated by the user prior to running samples with column. In the test report evaluating the performance of the column, chromatographic data are listed. These may include retention times of the components in the text mix, corresponding Kovats retention indices of several, if not all, of the solutes, the number of theoretical plates N and/or the effective plate number N_{eff}, Trennzahl and the acid/base inertness ratio (the peak height ratio of the acidic and basic probes in the test mixture). The values of two additional chromatographic parameters, separation number (Trennzahl number) and coating efficiency, may also be included in the report; the significance of TZ has been discussed in Section 3.6.4, and coating efficiency is treated in Section 3.10.5.

The first chromatogram obtained on a new column may be viewed as the "birth certificate" of a column and defines column performance at time $t = 0$ in the laboratory; a test mix should also be analyzed periodically to determine any changes in column behavior occurring with age and use. For example, a column may acquire a pronouncedly basic character if it has been employed routinely for amine analyses. Another important but often overlooked aspect is that a test mixture serves to monitor the performance of the *total* chromatographic system, not just the performance of the column. If separations gradually deteriorate over time, the problem may not always be column-related but could be due to extra-column effects, such as a contaminated or activated inlet liner. Commonly used components, their accepted abbreviations, and functions are listed in Table 3.16.

An ideal capillary column should be well deactivated and have excellent thermal stability and high separation efficiency. The extent of deactivation is usually

TABLE 3.16 Test Mixture Components and Role

Probe	Function
n-Alkanes, typically C10–C15	Column efficiency; Trennzahl number (TZ)
Methyl esters of fatty acids, usually C9–C12 (E9–E12)	Separation number; column efficiency
1-Octanol (ol)	Detection of hydrogen-bonding sites, silanol groups
2,3-Butanediol (D)	More rigorous test of silanol detection
2-Octanone	Detection of activity associated with Lewis acids
Nonanal(al)	Aldehyde adsorption other than via hydrogen bonding
2,6-Dimethylphenol (P)	Acid–base character
2,6-Dimethylaniline (A)	Acid–base character
4-Chlorophenol	Acid–base character
n-Decylamine	Acid–base character
2-Ethylhexanoic acid (S)	More stringent measure of irreversible adsorption
Dicyclohexylamine (am)	More stringent measure of irreversible adsorption

Note: Abbreviations of the components in the comprehensive Grob mix indicated in parentheses.

manifested by the amount of peak tailing for polar compounds. The most comprehensive and exacting test mixture is the solution reported by Grob et al. (96) and is more sensitive to residual surface activity than other polarity mixes. Adsorption may cause (1) broadened peaks of Gaussian shapes, (2) a tailing peak of more or less the correct peak area, (3) a reasonably shaped peak with reduced area, and (4) a skewed peak of correct area but having an increased retention time. Furthermore, irreversible adsorption cannot always be detected by peak shape. In the Grob procedure one measures peak heights as a percentage of that expected for complete and undistorted elution. The technique encompasses all types of peak deformations (broadening, tailing, and irreversible adsorption). A solution whose components are present at specific concentrations is analyzed under recommended column temperature programming conditions.

In practice, the percentage of the peak height is determined by drawing a line (the 100% line) connecting the peak maxima of the nonadsorbing peaks (*n*-alkanes and methyl esters), as shown in Figure 3.24. Alcohols are more sensitive than the other probes to adsorption caused by hydrogen bonding to exposed silanols. The acid and base properties are ascertained with probe solutes such as 2,6-dimethylaniline and 2,6-dimethylphenol, respectively. However, most column manufacturers recommend a modification of the Grob scheme to circumvent the lengthy time involved and, instead, tailor the composition of the mix and column temperature conditions to be commensurate with the particular deactivation procedure and stationary phase under consideration. A widely used test mixture consists of the components designated in Figure 3.25, where the test mix is used to also demonstrate selectivity by comparing separations on the three columns of the same dimensions but having stationary phases.

Guthrie and Harland (4) have commented that the effects of deactivation, the chemistry of the stationary phase and its crosslinking, as well as the effect of any postprocess treatment all appear in the final version of a column. An example of this situation is the separation of the Grob mixture (Figure 3.26a) performed on

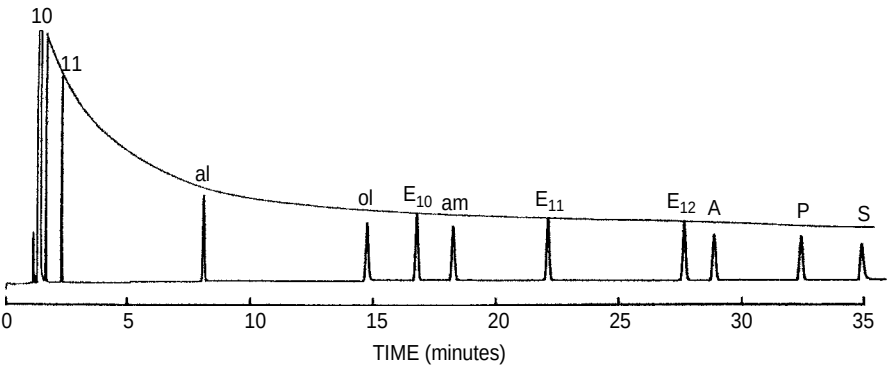


FIGURE 3.24 Chromatogram of a comprehensive Grob mixture on a 15-m × 0.32-mm-i.d. Carbowax 20M capillary column. Column conditions: 75–150°C at 1.7°C/min; 28 cm/s He. Designation of solutes appears in Table 3.16. (Reference 7.)

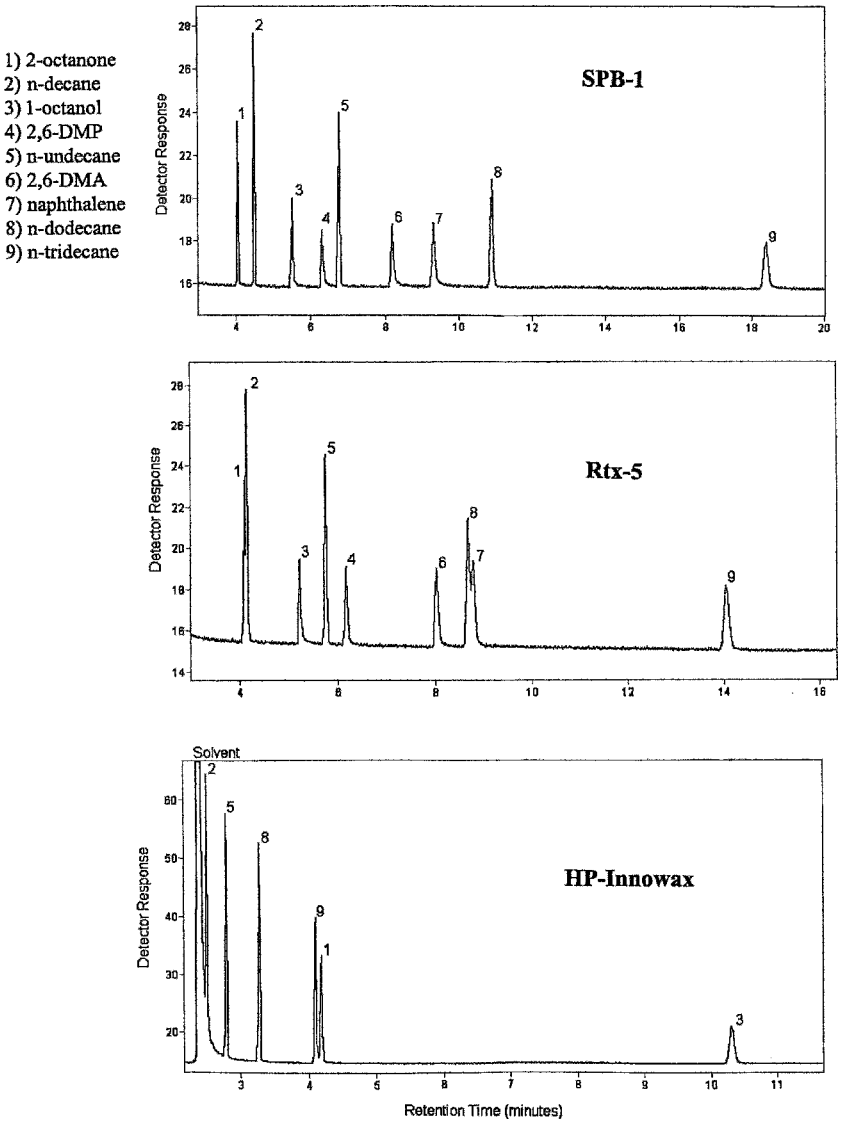


FIGURE 3.25 Chromatograms of an activity mixture on three columns of identical dimensions but different stationary phases as indicated; conditions: 15-m × 0.25-mm-i.d. × 0.25-μm-film capillary columns, 110°C, 25 cm/s He, FID.

a 15-m × 0.25-mm-i.d. fused-silica capillary column deactivated with Carbowax 20M, after which the column received a recoat of the polymer. After crosslinking of the stationary phase (Figure 3.26b), column behavior changed markedly. The 2,3-butanediol peak (D), absent in Figure 3.26a, is present on the crosslinked phase that has acquired increased acidity in the crosslinking process. Note the

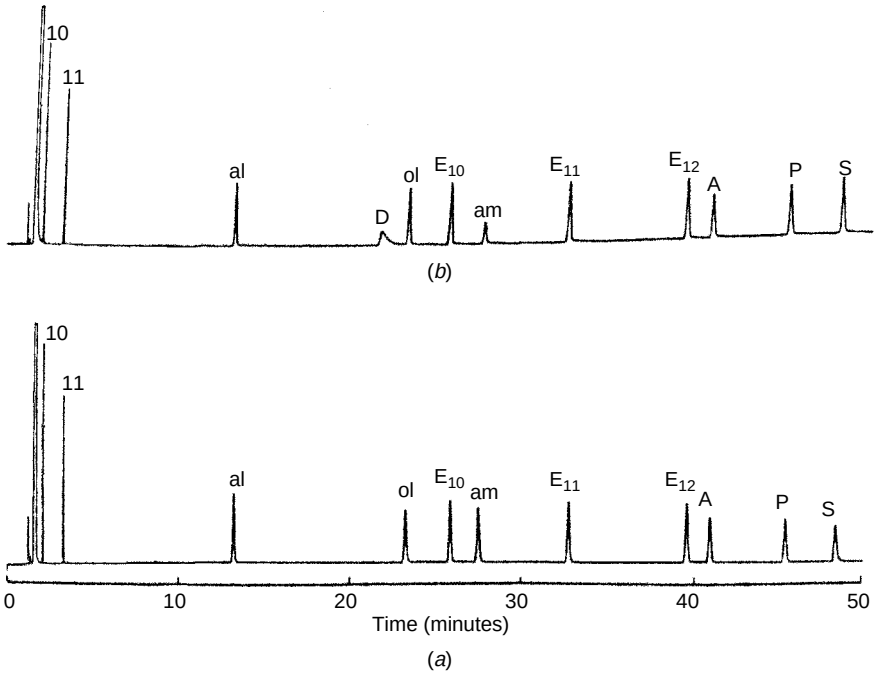


FIGURE 3.26 Chromatogram of a comprehensive Grob mixture on a 15-m × 0.32-mm-i.d. Carbowax 20M capillary column (a) after coating and (b) after crosslinking the stationary phase. Column conditions: 75–150°C at 2°C/min; 28 cm/s He. Designation of solutes appears in Table 3.16. (Reference 7.)

decreased peak height of the dicyclohexylamine probe (am) and the increased peak height of 2-ethylhexanoic acid (S). Thus, any change or a minor modification in column preparation can affect the final column performance.

3.10 CHROMATOGRAPHIC PERFORMANCE OF CAPILLARY COLUMNS

3.10.1 Golay Equation versus van Deemter Expression

The fundamental equation underlying the performance of a gas chromatographic column is the van Deemter expression (Equation 2.44; further discussed in Section 3.6.4), which may be expressed as

$$H = A + \frac{B}{\overline{u}} + C\overline{u} \tag{3.26}$$

where H = height equivalent to a theoretical plate
A = eddy diffusion or multiple path term
B = longitudinal diffusion contribution
C = resistance to mass transfer term
$\overline{u}$ = average linear velocity of carrier gas

In the case of a capillary column, the A term is equal to zero because there is no packing material. Thus, Equation (3.26) simplifies to

$$H = \frac{B}{\overline{u}} + C\overline{u} \tag{3.27}$$

This abbreviated expression is often referred to as the *Golay equation* (43). The B term may be expressed as $2D_g/\overline{u}$, where D_g is the binary diffusion coefficient of the solute in the carrier gas. Peak broadening due to longitudinal diffusion is a consequence of the residence time of the solute within the column and the nature of the carrier gas. This effect becomes pertinent only at low linear velocities or flowrates and is less pronounced at high velocities (Figures 2.20 and 2.21).

However, the major contributing factor contributing to band broadening is the C term, in which the resistance to mass transfer can be represented as the composite of the resistance to mass transfer in the mobile phase C_g and that in the stationary phase C_l:

$$C = C_g + C_l \tag{3.28}$$

where

$$C_g = \frac{r^2(1 + 6k + 11k^2)}{D_g 24(1 + k)^2} \tag{3.29a}$$

$$C_l = \frac{2kd_f^2\overline{u}}{3(1 + k)^2 D_l} \tag{3.29b}$$

where D_l is the diffusion coefficient of the solute in the stationary phase, k is the retention factor of the solute, d_f is the film thickness of the stationary phase, and r is the radius of the capillary column. With capillary columns, C_l is small and becomes significant only with capillary columns having a thick stationary-phase film. The Golay equation may then be rewritten as

$$H = \frac{B}{\overline{u}} + C_g\overline{u} = \frac{2D_g}{\overline{u}} + \frac{r^2(1 + 6k + 11k^2)\overline{u}}{D_g 24(1 + k)^2} \tag{3.30}$$

The optimum linear velocity corresponding to the minimum in a plot of H versus u (Figure 2.20) can be obtained by setting $dH/du = 0$ and solving for $\overline{u}$:

$$\frac{dH}{du} = 0 = \frac{B}{\overline{u}^2} + C_g \tag{3.31}$$

Thus, $u_{opt} = (B/C_g)^{1/2}$ and the value of H corresponding to this optimum linear velocity H_{min} *is*

$$H_{min} = r\sqrt{\frac{1 + 6k + 11k^2}{3(1 + k)^2}} \tag{3.32}$$

Consequently, as the diameter of a capillary column decreases, both maximum column efficiency N and maximum effective efficiency N_{eff} increase and are also dependent on the particular solute retention k. Retention in capillary GC is usually expressed as, the retention factor k, where

$$k = (t_R - t_M)/t_M.$$

In Table 3.17 the effect of column inner diameter on maximum attainable column efficiency N is presented as a function of retention factor. For a capillary of a given inner diameter, one can see that there is an increase in plate height with increasing k, with a corresponding decrease in plate number, and an increase in effective plate count. As the inner diameter of a capillary column increases, column efficiency N drops markedly, while the effective plate number N_{eff} increases. For separations requiring high resolution, columns of small inner diameter are recommended. Expressing efficiency in terms of plates per meter allows the efficiency of columns of unequal lengths to be compared. Also included in Table 3.17 are data for 0.53 mm i.d., the diameter of the "megabore" capillary column, which has been designated as the alternative to the packed column. The merits and features of this particular type of column are discussed in Section 3.10.4.

3.10.2 Choice of Carrier Gas

Capillary-column efficiency is dependent on the carrier gas used, the length and inner diameter of the column, the retention factor of the particular solute selected for the calculation of the number of theoretical plates, and the film thickness of stationary phase. Profiles of H versus u for three carrier gases with a thin-film capillary column are displayed in Figure 3.27. Although the lowest minimum and, therefore, the greatest efficiency are obtained with nitrogen, speed of analysis must be sacrificed, as is shown in Figure 3.28 (93). The increasing portion of the curve is steeper for nitrogen in Figure 3.27, which necessitates working at or near u_{opt}; otherwise, loss in efficiency (and resolution) quickly results. On the other hand, if one is willing to accept a slight loss in the number of theoretical plates, a more favorable analysis time is possible with helium and hydrogen as carrier gases, because u_{opt} occurs at a higher linear velocity. Moreover, the mass transfer contribution or rising portion of a curve is less steep with helium or hydrogen, which permits working over a wider range of linear velocities without substantial sacrifice in resolution. This advantage becomes evident in comparing the capillary separation of the components in calmus oil with nitrogen and hydrogen as carrier gases in Figure 3.29.

TABLE 3.17 Column Efficiency as a Function of Inner Diameter and Retention Factor

Inner Diameter (mm)	k	h_{min}	Maximum Plates per Meter, N	Effective Plates per Meter, N_{eff}
	1	0.061	16,393	4,098
	2	0.073	13,697	6,027
	5	0.084	11,905	6,667
0.10	10	0.090	11,111	9,222
	20	0.093	10,752	9,784
	50	0.095	10,526	10,105
	1	0.153	6,536	1,634
	2	0.182	5,495	2,442
	5	0.210	4,762	3,307
0.25	10	0.224	4,464	3,689
	20	0.231	4,329	3,925
	50	0.236	4,237	4,073
	1	0.196	5,102	1,276
	2	0.232	4,310	1,896
	5	0.269	3,717	2,082
0.32	10	0.286	3,497	2,903
	20	0.296	3,378	3,074
	50	0.302	3,311	3,179
	1	0.325	3,076	769
	2	0.384	2,604	1,146
	5	0.445	2,247	1,258
0.53	10	0.474	2,110	1,751
	20	0.490	2,041	1,857
	50	0.500	2,000	1,920

In comparing these carrier gases, another benefit becomes apparent at linear velocities corresponding to equal values of plate height. With the lighter carrier gases solutes can be eluted at lower column temperatures during temperature programming with narrower band profiles, since higher linear velocities can be used. Thus, either helium or hydrogen is recommended over nitrogen and indeed these gases are used today as carrier gases for capillary gas chromatography. One advantage of using hydrogen is that plate number varies less for hydrogen than for helium as linear velocity increases. The use of hydrogen for any application in the laboratory always requires safety precautions in the event of a leak. Precautionary measures should be taken for the safe discharge of hydrogen from the split vent in the split-injection mode.

3.10.2.1 Measurement of Linear Velocity

The flowrate through a capillary column whose inner diameter is less than 0.53 mm is difficult to measure accurately and reproducibly by a conventional

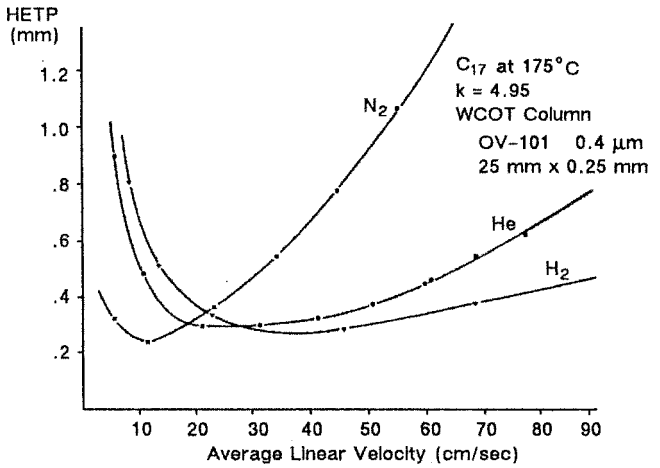


FIGURE 3.27 Profiles of HETP versus linear velocity for the carrier gases: helium, hydrogen, and nitrogen; courtesy of Agilent Technologies.

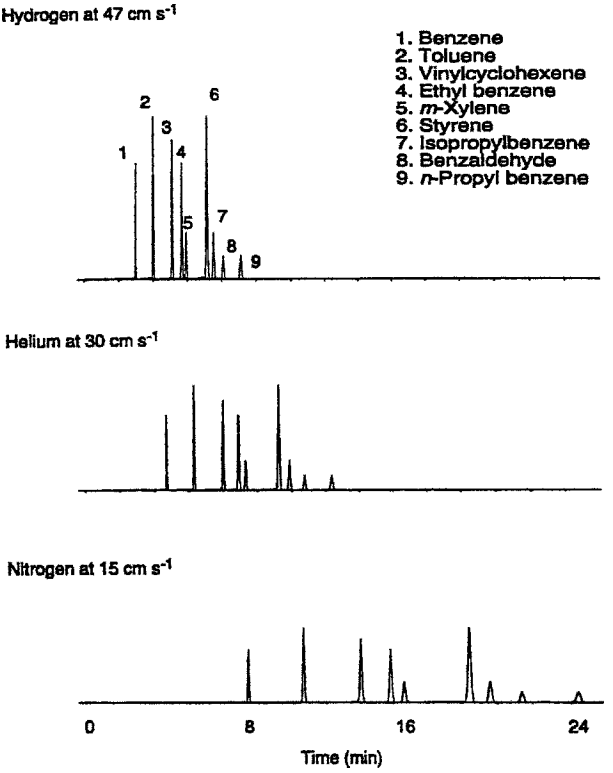


FIGURE 3.28 Effect of carrier gas on separation at optimum linear velocities (reproduced from Reference 93: D. W. Grant, in *Capillary Gas Chromatography*, copyright 1996, John Wiley & Sons Limited; reproduced with permission).

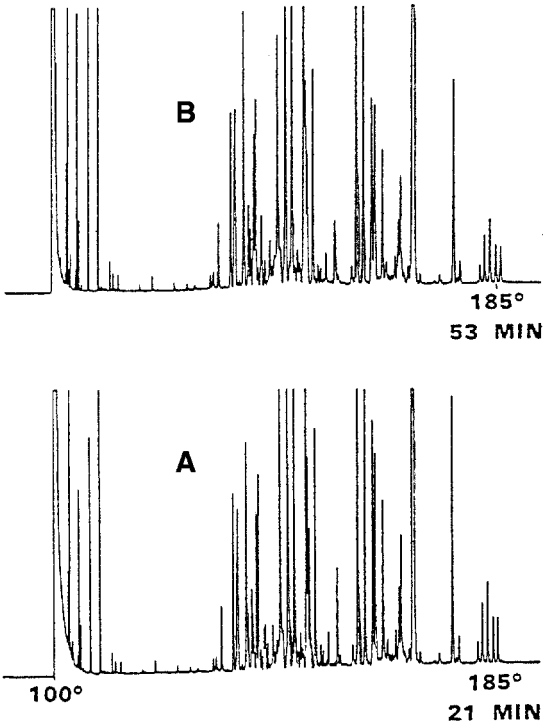


FIGURE 3.29 Chromatograms of the separation of calmus oil using (a) hydrogen as carrier gas, 4.2 mL/min at a programming rate of 4.0°C/min and (b) nitrogen as carrier gas, 2.0 mL/min programming rate of 1.6°C/min on a 40-m × 0.3-mm-i.d. capillary column (0.12-μm film) (reproduced from Reference 97 and reprinted with permission from Dr. Alfred Huethig Publishers).

soap-bubble flowmeter. Instead, the flow of carrier gas through a capillary column is usually expressed as linear velocity rather than as a volumetric flowrate. Linear velocity may be calculated by injecting a volatile, nonretained solute and noting its retention time t_M (seconds). For a capillary column of length L in centimeters, we obtain

$$u(\text{cm/s}) = \frac{L}{t_M} \tag{3.33}$$

For example, the linear velocity of carrier gas through a 30-m column where methane has a retention time of 2 min is 3000 cm/120 s or 25 cm/s. If desired, the volumetric flowrate F (mL/min) can be computed from the relationship

$$F(\text{mL/min}) = 60\pi r^2 u \tag{3.34}$$

where r is the radius of the column in centimeters. An injection of methane is convenient to use with a FID to determine t_M and/or a headspace injection

of methylene chloride and acetonitrile can be made with an ECD and NPD, respectively. Nitrogen and oxygen (air) may be used with a MS while ethylene or acetylene vapors can be injected with a PID. Recommended linear velocities and flowrates of helium and hydrogen for capillary columns of various inner diameters are listed in Table 3.18.

3.10.2.2 Effect of Carrier-Gas Viscosity on Linear Velocity

Chromatographic separations using capillary columns are achieved under constant pressure conditions, as opposed to packed columns, which are usually operated in a flow-controlled mode. The magnitude of the pressure drop across a capillary column necessary to produce a given linear velocity is a function of the particular carrier gas and length/inner diameter of the column. The relationship between viscosity and temperature for any gas is linear, as shown in Figure 3.30 for helium, hydrogen, and nitrogen. In gas chromatography, as column temperature increases, linear velocity decreases because of increased viscosity of the carrier gas. Thus, initially higher linear velocities are established for temperature-programmed analyses than for isothermal separations. If we compare columns of identical dimensions and operate them at the same inlet pressure and temperature, the linear velocity will be highest for hydrogen and lowest for helium. Therefore, whenever a change in the type of carrier gas is made in the laboratory, linear velocities should actually be measured and one should not reconnect the pressure regulator using the same delivery pressure.

3.10.3 Phase Ratio

In addition to the nature of the carrier gas, column efficiency and, ultimately, resolution and sample capacity of a capillary column are affected by the physical nature of the column, namely, the inner diameter and film thickness of stationary phase. An examination of the distribution coefficient K_D as a function of chromatographic parameters is helpful here. K_D is constant for a given

TABLE 3.18 Recommended Linear Velocities and Flowrates With Helium and Hydrogen

Inner Diameter	Linear Velocity (cm/s)		Flowrate (mL/min)	
(mm)	Helium	Hydrogen	Helium	Hydrogen
0.18	20–45	40–60	0.3–0.7	0.6–0.9
0.25	20–45	40–60	0.7–1.3	1.2–2.0
0.32	20–45	40–60	1.2–2.2	2.2–3.0
0.53	20–45	40–60	4.0–8.0	6.0–9.0

Note: 30-m column length.

Source: Data abstracted from 1994/95 J&W Scientific Catalog.

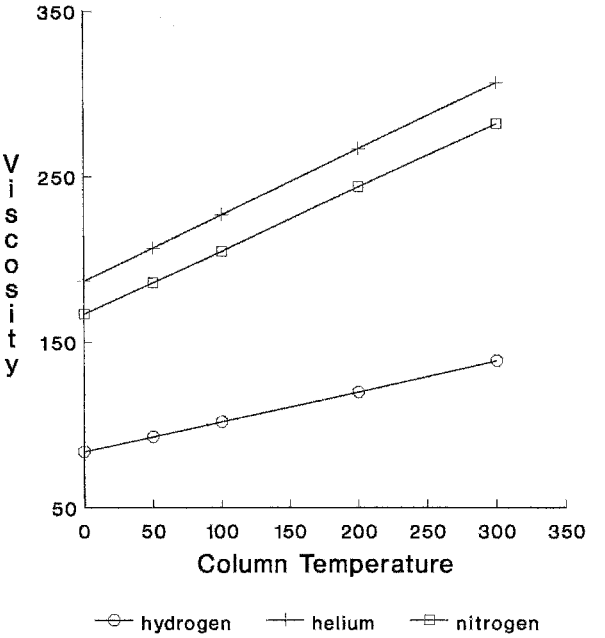


FIGURE 3.30 Effect of temperature on carrier-gas viscosity. (Data for curves generated from viscosity-temperature relationships in Reference 83.)

solute–stationary-phase pair and is dependent only on column temperature. K_D may be defined as

$$K_D = \frac{\text{concentration of solute in stationary phase}}{\text{concentration of solute in carrier gas}} \tag{3.35}$$

or

$$K_D = \frac{\text{amount of solute in stationary phase}}{\text{amount of solute in mobile phase}} \times \frac{\text{volume of carrier gas}}{\text{volume of stationary phase in column}} \tag{3.36}$$

K_D can now be expressed as

$$K_D = k\beta = k\frac{r}{2d_f} \tag{3.37}$$

where β is the phase ratio and is equal to $r/2d_f$, r is the radius of the column, and d_f is the film thickness of the stationary phase. At a given column temperature, retention increases as the phase ratio of the column decreases, which can be manipulated either by decreasing the diameter of the column or increasing the

film thickness of the stationary phase; likewise a decrease in retention is noted with an increase in β. Since K_D is a constant at a given column temperature, film thickness and column diameter play key roles in determining separation power and sample capacity. In selecting a capillary column, the phase ratio should be considered.

As the film thickness decreases, *k* or retention factor also decreases at constant temperature, column length, and inner diameter. Conversely, with an increase in film thickness in a series of columns having the same dimensions, retention increases under the same temperature conditions. This effect of film thickness on separation is demonstrated in the series of parallel chromatograms appearing in Figure 3.31. Column diameter limits the maximum amount of stationary phase that can be coated on its inner wall. Small-diameter columns usually contain thinner films of stationary phase, while thicker films can be coated on wider-bore columns. The concept of phase ratio allows two columns of equal length to be compared in terms of sample capacity and resolution.

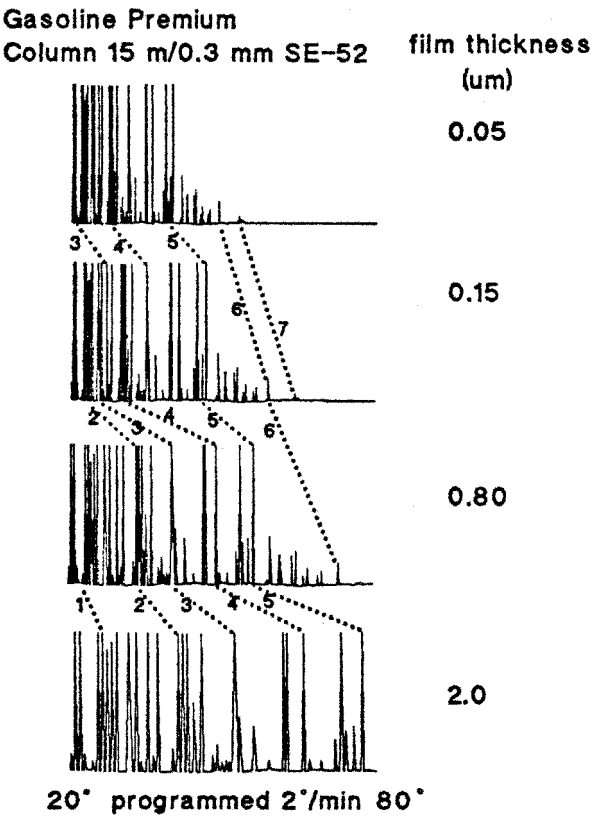


FIGURE 3.31 Chromatograms of gasoline on capillary columns with varying film thickness of stationary phase, SE-52 (reproduced from Reference 97 and reprinted with permission of Dr. Alfred Huethig Publishers).

As depicted in Table 3.17, column efficiency increases as column diameter decreases. Sharper peaks yield improved detection limits. However, as column diameter decreases, so does sample capacity. Column temperature conditions and linear velocity of the carrier gas can usually be adjusted to have a more favorable time of analysis. In Figure 3.32 these parameters are placed into perspective in a pyramidal format as a function of the inner diameter of a capillary column.

3.10.4 Practical Considerations of Column Diameter, Film Thickness, and Column Length

Guidelines for the selection of column diameter, film thickness of stationary phase, and length will now be established on the basis of practical gas chromatographic considerations.

3.10.4.1 Column Diameter

1. Sample capacity increases as column diameter increases. Samples having components present in the same concentration range can be analyzed on a column of any diameter. The choice is dependent on resolution required.

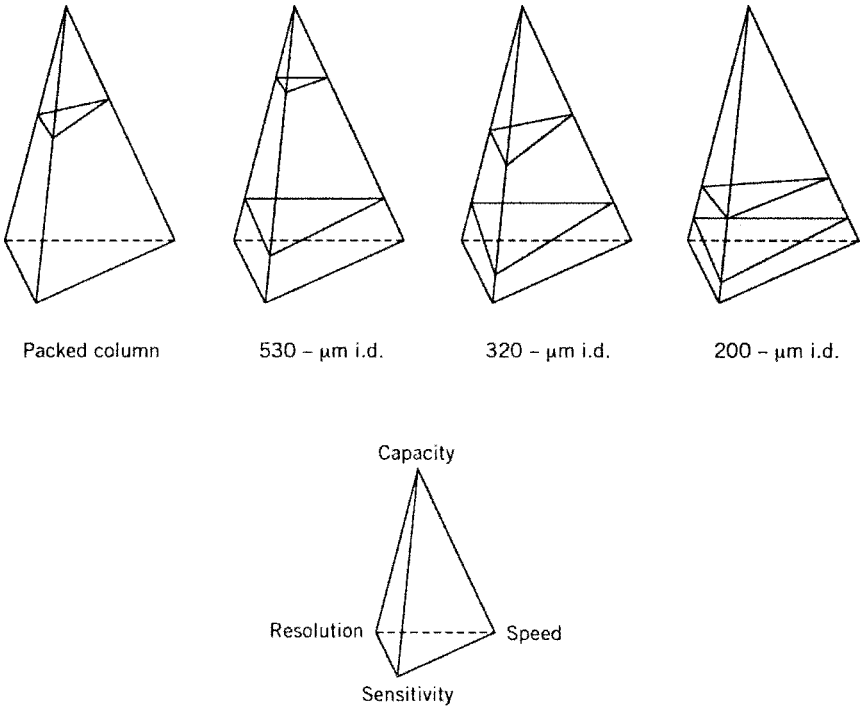


FIGURE 3.32 The "chromatographic pyramids" for packed and capillary columns of varying inner diameter (courtesy of Agilent Technologies).

In general, sample capacity of any capillary column is proportional to the square of the column radius.

2. For complex samples, select a column having the smallest diameter and sample capacity compatible with the concentration range of the sample components.
3. Samples whose components differ widely in concentration should be analyzed on a column of larger i.d. (>0.25 mm) to avoid overload of the column by solutes of higher concentration.
4. The selection of column i.d. may be based on the type of sample inlet system. Generally, a 0.25- or 0.32-mm-i.d. column may be used for split and splitless injections, 0.32 mm i.d. for splitless and on-column injections, and 0.53 mm i.d. for direct injections.
5. Capillary columns of 0.18 and 0.25 mm i.d. should be used for GCMS systems, because the lower flowrates with these columns will not exceed the limitations of the vacuum system.
6. Fast capillary columns (0.10 mm i.d.) are used for rapid analyses because the same resolution can be generated in a shorter time.
7. The square root of resolution is proportional to column i.d. The smaller the i.d. the greater will be column efficiency; the shorter will be the time of analysis for a specific degree of resolution.

3.10.4.2 Film Thickness of Stationary Phase

1. Retention and sample capacity increase with increasing film thickness with a concurrent decrease in column efficiency.
2. Film thickness is inversely proportional to plate number and almost directly proportional to time of analysis.
3. Thin-film columns provide higher resolution of high-boiling solutes but lower resolution of more volatile components under any set of column temperature conditions.
4. The sample capacity of thin-film columns may be inadequate and require cryogenic temperature control of the column oven.
5. Film thicknesses of <0.2 μm permit the use of longer columns for complex samples.
6. A solute will exhibit a lower elution temperature as film thickness decreases; thus, thin-film columns are ideal for high-boiling petroleum fractions, triglycerides, and other compounds.
7. A thick-film column (which inherently are more inert) should be utilized for samples having a range of solute concentrations. Thicker films of stationary phase (>1 μm) should be used for analysis of more volatile solutes. Very thick films (>5 μm) should be selected for analyses to be performed at room temperature.
8. Thicker-film columns necessitate higher elution temperatures, but incomplete elution of all sample components may result.

9. Higher elution temperatures for prolonged periods of time mean a reduced column lifetime and more column bleed.
10. A capillary column, 30 m or longer, with a thick film of stationary phase, offers an alternative to cryogenic oven temperature control for solute-focusing purposes, which is especially attractive with auxiliary sample introduction techniques of purge and trap and thermal desorption.

Sample capacities for capillary columns of several inner diameters with different film thicknesses are summarized in Table 3.19.

3.10.4.3 Column Length

Resolution is a function of the square root of the number of theoretical plates or column length. One must consider the tradeoff of the increase in overall resolution in a separation by augmenting column length with the simultaneous increase in analysis time under isothermal conditions. Prudence suggests using the shortest column length that will produce the necessary resolution. The sample capacity of a capillary column increases with column length. Increasing the length of a capillary column from 15 to 30 m, for example, results in an improvement by a factor of 1.4 (the square root of 2) in resolution, but analysis time also doubles (Equation 3.15), which may limit sample throughput in a laboratory. To double resolution between two adjacent peaks, one needs a fourfold increase in column length. If one is already using a 30-m column, increasing the column length to 120 m is unreasonable. Here a column of the same initial length (or shorter sometimes) having another stationary phase will have a different selectivity and solve the problem. The situation is slightly different under temperature programming

TABLE 3.19 Column Capacity as a Function of Inner Diameter and Film Thickness

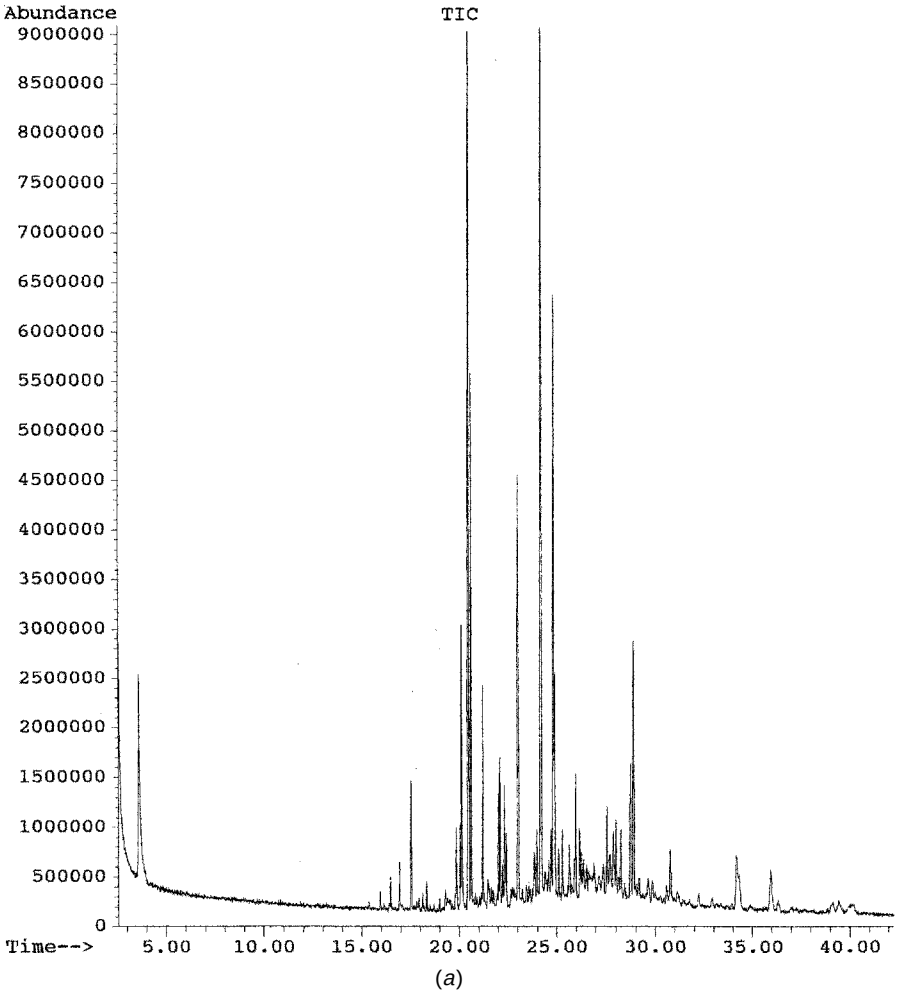
Inner Diameter (mm)	Film Thickness (μm)	Capacity[a] (ng/component)
0.25	0.15	60–70
	0.25	100–150
	0.50	200–250
	1.0	350–400
0.32	0.25	150–200
	0.5	250–300
	1.0	400–450
	3.0	1200–1500
0.53	1.0	1000–1200
	1.5	1400–1600
	3.0	3000–3500
	5.0	5000–6000

[a] *Capacity* is defined as the amount of component where peak asymmetry occurs at 10% at half-height.

Source: Data abstracted from 1994/95 J&W Scientific Catalog.

conditions where a large improvement in resolution can sometimes be obtained with only a moderate increase in analysis time.

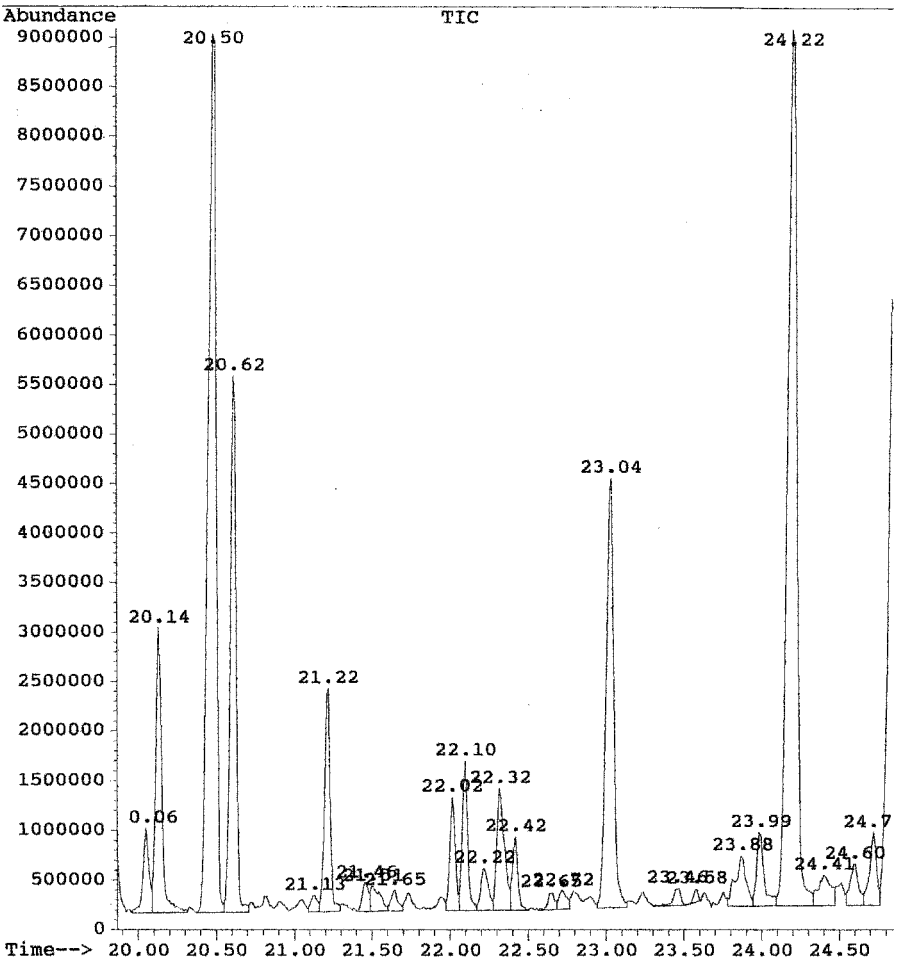
The best approach is the selection of a 25- or 30-m column for general analytical separations (Figure 3.33) and for fingerprinting chromatograms generated under the same chromatographic conditions for comparison of samples (Figure 3.34). A shorter length of column may be employed for rapid screening



(*a*)

FIGURE 3.33 (a) Total-ion chromatogram of a chloroform extract of a wood sliver from a telephone pole for determination of pentachlorphenol; (b) expanded view of chromatogram ranging from 20 to 25 min. Peak at retention time of 20.14 min was determined to be pentachlorphenol. Conditions: 30-m × 0.25-mm-i.d. DB-5 column with 0.25-μm film. Column conditions: 40°C at 8°C/min to 250°C after 1 min isothermal hold; splitless injection of 1 μL (1 min delay time).

```
File       : D:\HPCHEM\1\DATA\C02.D
Operator   : Pakorn
Acquired   : 18 Nov 98    6:43 pm using AcqMethod GROBTEMP
Instrument :   5971 - In
Sample Name: C23691-wood
Misc Info  : Splitless, 1uL, SCAN
Vial Number: 1
```



(*b*)

FIGURE 3.33 (*Continued*)

or simple mixtures or a 60-m column for very complex samples (a longer column also generates more column bleed). Temperature programming ramp profiles can be adjusted to optimize resolution. A number of studies dealing with computer simulation based on optimization of column temperature have been reported (98–102).

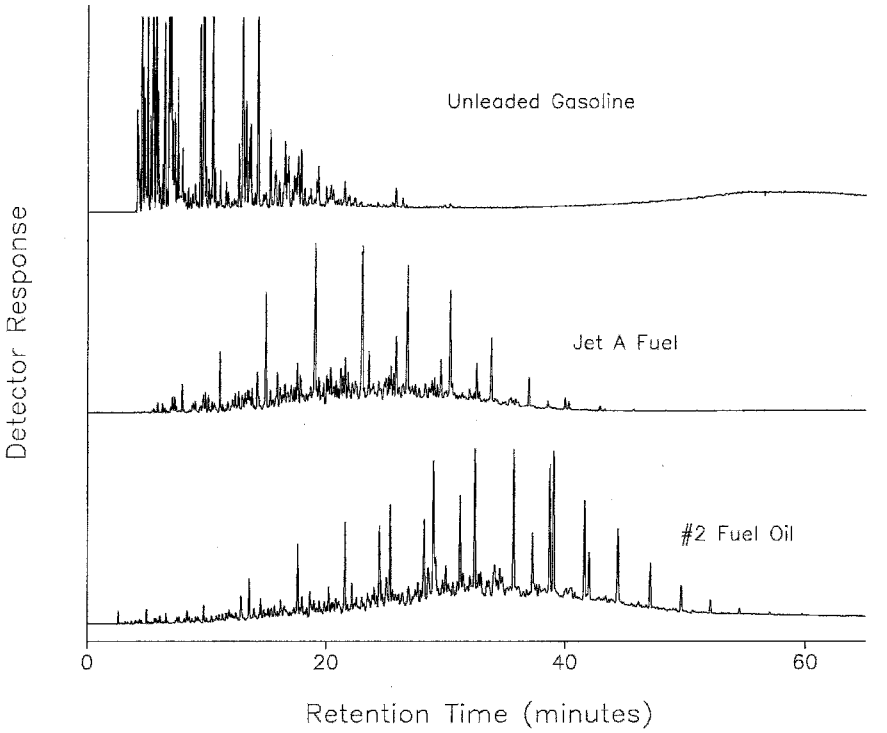
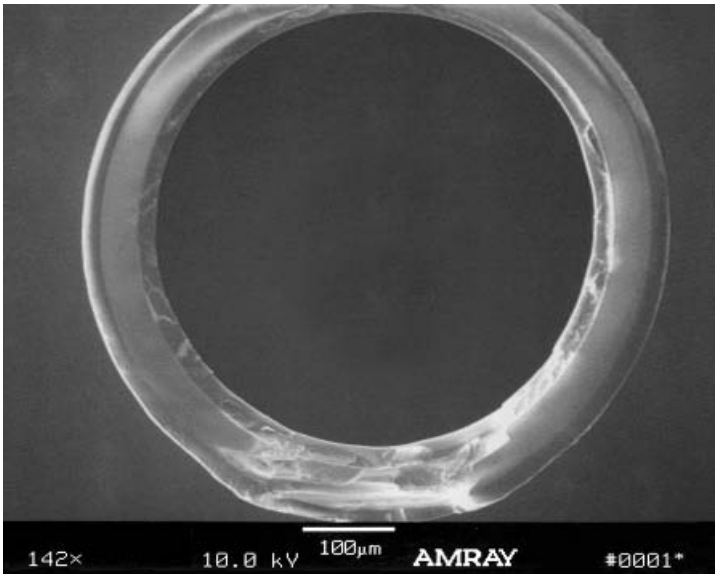


FIGURE 3.34 Chromatograms showing the separation of an unleaded gasoline, jet A fuel and No. 2 fuel oil under the same column conditions. Column: 30-m × 0.25-mm-i.d. HP-1 (0.25-μm film); temperature conditions 35°C (2 min) at 4°C/min to 260°C. Det: FID, 25 cm/s He.

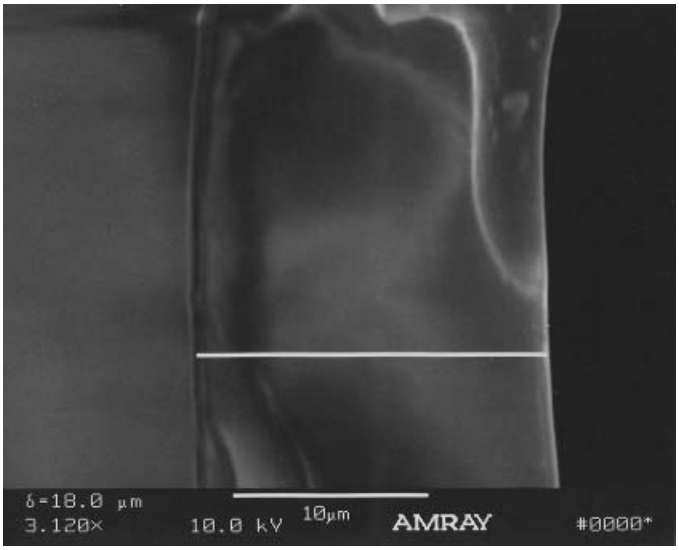
3.10.4.4 Capillary Columns of 0.53 mm i.d. (The Megabore Column)

Many applications previously performed on a packed column can now be done with a megabore column, a capillary column of 0.53 mm i.d. A megabore column with a fairly thick film of stationary phase has a low phase ratio like a packed column and exhibits retention characteristics and sample capacities similar to those of a packed column. For example, the phase ratios of a 0.53 mm i.d. column with film thicknesses of 3.00 and 5.00 μm are 44 and 26, respectively, and lie in the range of the phase ratio of a packed column. An interesting scanning electronmicrograph (SEM) of a cross section of a capillary column having a very thick film of stationary phase (~18 μm) appears in Figure 3.35. Examination of the photograph indicates the film thickness of stationary phase is nearly identical to the thickness of the polyimide outer coating on this particular column.

A 0.53-mm-i.d. column offers the best of both worlds, because it combines the attributes of a fused-silica capillary column with the high sample capacity and ease of use of a packed column. Analytical methods developed with a packed column can be easily transferred for many applications to a megabore column



(*a*)



(*b*)

FIGURE 3.35 Scanning electronmicrograph of the crossection of a 0.53-mm-i.d. column; the 18-µm stationary-phase film thickness is approximately equal to the thickness of the polyimide outer coating on the capillary (SEM photographs courtesy of the Quadrex Corp.)

with the appropriate stationary phase. Peaks generated with a megabore column typically are sharper and exhibit less tailing compared to those with a packed column. Redistribution of the stationary phase can occur at the inlet of packed column with large injections of solvent and leave an exposure of silanol sites on a diatomaceous earth support. With a crosslinked phase in a megabore column, this problem is eliminated. Lewis acid sites, which are a problem with supports, are likewise absent in this larger-diameter capillary column. Analysis time is also shorter as a rule (Figure 3.36). On the other hand, long megabore columns can

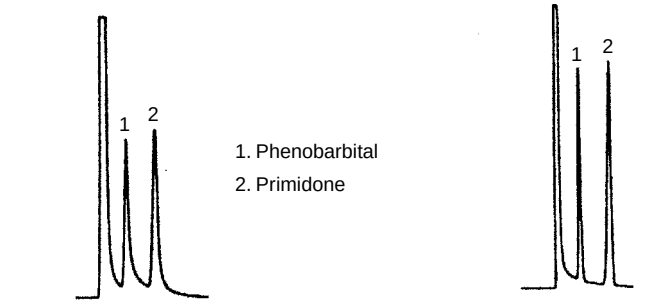


FIGURE 3.36 Chromatograms comparing (a) the effect of the inertness of a 0.53-mm-i.d. column to the more active surfaces within a packed column and (b) the retention characteristics of a packed column and 0.53-mm-i.d. column (courtesy of Agilent Technologies).

be used for the analysis of more complex samples, such as the separation of the reference standard for EPA Method 502.2 (VOCs in drinking water) presented in Figure 3.37.

3.10.5 Coating Efficiency

This parameter, also called the utilization of theoretical efficiency (UTE), is the ratio of the actual efficiency of a capillary column to its theoretical maximum possible efficiency. Coating efficiency or UTE is expressed as

$$\% \text{ Coating efficiency} = \frac{H_{\min}}{H} \times 100 \tag{3.38}$$

where $H_{\min}$ is as defined in Equation 3.11. Coating efficiency is a measure of how well a column is coated with stationary phase. Coating efficiencies of nonpolar columns range from 90 to 100%. Polar columns have somewhat lower coating

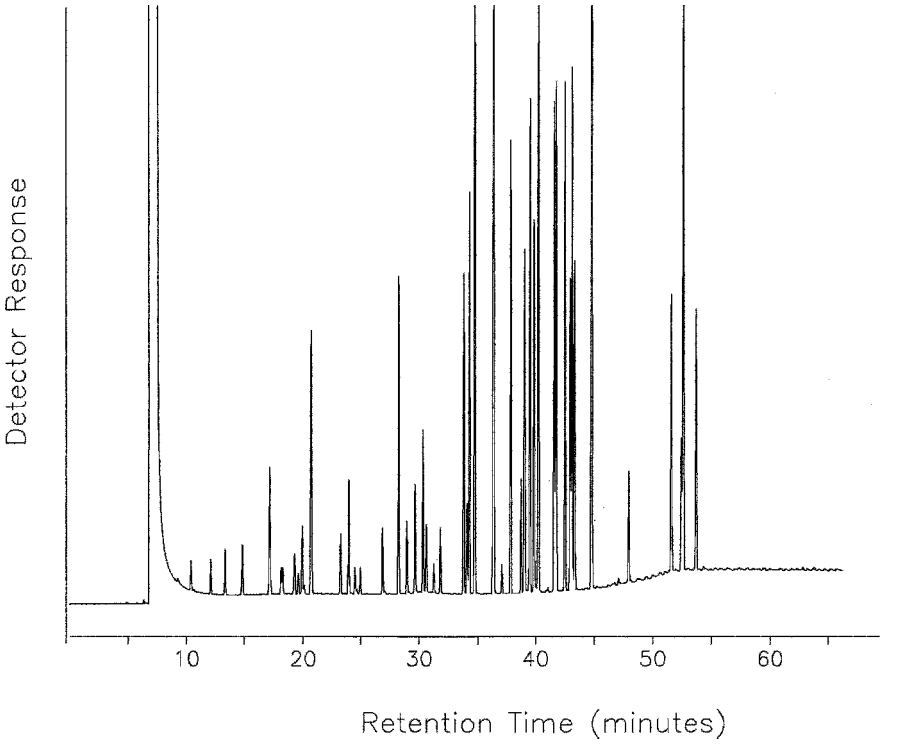


FIGURE 3.37 Chromatogram of the separation of reference standard for EPA Method 502.2 on a 75-m × 0.53 mm-i.d. methyphenylcyanopropylsilicone capillary column (2.5-μm film). Column conditions: 35°C at 4°C/min to 280°C after 10 min isothermal hold; 10 mL/min He; splitless injection and FID.

efficiencies, 60–80%, because a polar stationary phase is more difficult to coat uniformly. Also, larger-diameter columns tend to have higher coating efficiencies. This parameter is typically listed on a test report shipped with a new column and is important to column manufacturers for monitoring the quality of their product. It is usually of no concern to most capillary column users.

3.11 STATIONARY-PHASE SELECTION FOR CAPILLARY GAS CHROMATOGRAPHY

3.11.1 Requirements and History

The use of packed columns for gas chromatographic separations requires having an assortment of columns available with different stationary phases in order to compensate for column inefficiency by a commensurate gain in selectivity. With a capillary column, the demands on selectivity, although important, are not as stringent, because of the high plate count possible with a capillary column. However, with the transition from the era of the packed to the capillary column, a gradual redefinement in the requirements of the stationary phase took place.

Many liquid phases for packed-column purposes were unacceptable for capillary GC. Although they offered selectivity, overriding factors responsible for their disfavor were overall lack of thermal stability and the instability of the stationary phase as a thin film at elevated temperatures and during temperature programming. In the latter processes, it is crucial that the phase remain a thin uniform film; otherwise, loss of both inertness and column efficiency result. Today, these problems have been solved and the refinements are reflected in the high performance of commercial columns. The impetus has been driven by the improvements in the sensitivity of mass spectrometers such that the MS detector is now the second most popular detector in GC (the FID is the most widely used detector). This rise in the use of GCMS has also necessitated more thermally stabile columns offering much less column bleed.

Factors influencing the choice of inner diameter, film thickness of stationary phase, and column length have been discussed in Section 3.10.4. Let us now focus on selection of the stationary phase, the most important aspect in column selection.

In choosing a stationary phase for capillary separations, remember the adage "like dissolves like." As a starting point, try to match the functional groups present in the solutes under consideration with those in a stationary phase, as is the case in the selection of a packed column, of course. In the analysis of polar species, for instance, select a polar stationary phase. Fine-tune this choice, if necessary, by examining McReynolds constants of specific interactions of a particular solute with a stationary phase. However, for reasons that will be elucidated throughout the remainder of Section 3.11, a polar phase, when compared to a nonpolar one, tends to exhibit slightly less column efficiency, has a lower maximum temperature limit, and will have a shorter lifetime if operated for a prolonged period of time at an elevated temperature. The effect of the lower thermal stability of polar

phases can be alleviated by selecting a thinner film of stationary phase and a shorter column length for more favorable elution temperatures. Here are some guidelines:

1. Use the least polar phase that will generate the needed separation.
2. Nonpolar phases are more "forgiving," because they are more resistant to traces of oxygen and water in the carrier gas and basically oxidation and hydrolysis, generally more so than a polar phase.
3. Nonpolar phases are more inert, bleed less, have a wide range of operating column temperatures, and have higher coating efficiencies.
4. With nonpolar stationary phases, separations occur on the basis of boiling points for the most part. By introducing or increasing trifluoropropyl, cyanopropyl, or phenyl content, separations result from interactions between functional groups, dipoles, charge distributions, and other factors.
5. Separations of compounds that differ in their capacities for hydrogen bonding such as alcohols, and aldehydes can probably be best achieved with polyethylene glycol-type stationary phases.

The list of different stationary phases available for capillary separations appearing in Table 3.20 is certainly not an exhaustive one and includes the commonly used polysiloxanes and polyethylene glycol phases, which are suitable for most applications. The majority of analyses can be performed on columns containing 100% dimethyl polysiloxane or 5% phenyl–95% methylpolysiloxane, a cyanopolysiloxane, and a polyethylene glycol. Additional selectivity in a separation can always be improved or achieved by using a trifluoropropylpolysiloxane or phases of varying cyano and phenyl content. Separation of permanent gases and light hydrocarbons can now be performed on a capillary column containing an adsorbent (a porous polymer, alumina, molecular sieves), which serves as a direct substitute of the packed-column version.

The coating of a glass capillary column was achieved by roughening its inner surface prior to coating for enhanced wettability by stationary phases having a wide range of polarities and viscosities, but this option is unavailable with fused silica. The wettability of fused silica proved to be more challenging because its thin wall does not permit aggressive surface modification. Consequently, fewer phases initially could be coated on fused silica compared to glass capillaries. Although polar phases could be deposited successfully on glass capillaries, fused-silica columns coated with polar phases were especially inferior in terms of efficiency and thermal stability.

Viscosity of the film of stationary phase after deposition under the thermal conditions of GC proved to be an important consideration. Wright and coworkers (103) correlated viscosity of a stationary phase with coating efficiency and stability of the coated phase. The results of their study supported the experimental success of viscous gum phases, which yielded higher coating efficiencies and had greater thermal stability than did corresponding nonviscous counterparts. The popularity of the nonpolar polysiloxane phases is due in part to the

TABLE 3.20 Cross-reference of Columns From Manufacturers

	Manufacturer								
Stationery Phase	Alltech	Chrompack	HP	J&W	Perkin–Elmer	Quadrex	Restek	SGE	Supelco
				Polysiloxanes					
100% Dimethyl	AT-1 SE-30	CP Sil 5 CB	HP-1, Ultra-1 HP-101, PONA	DB-1, DB-1HT DB-Petro DB-Petro 100 DB-2887	PE-1	007-1	Rtx-1, MXT-1 Rtx-2887, MXT-2887	BP-1	SPB-1, SP-2100 SPB-1 sulfur Petrocol DH 50.2 Petrocol 2887 Petrocol EX, Petrocol 3710
5% Phenyl– 95% dimethyl	AT-5 SE-54	CP Sil 8 CB	HP-5, Ultra-2 PAS-5	DB-5, DB-5 HT DB-5 MS DB-5.625 SE-54	PE-2	007-2	Rtx-5, MXT-5 XTI-5	BP-5	SPB-5, PTE-5 SE-54
6% Cyanopropyl-phenyl– 94% dimethyl	— —	— —	HP-1301	DB-1301 DB-624	—	007-502	Rtx-1301 Rtx-624 MXT-624	—	—
20% Diphenyl– 80% dimethyl	—	—	—	—	PE-7	007-7	Rtx-20 MXT-20	— —	SPB-20 VOCOL
35% Diphenyl– 65% dimethyl	AT-35	—	HP-35	—	PE-11	007-11	Rtx-35	—	SPB-35 SPB-608
14% Cyanopropyl-phenyl– 86% dimethyl	AT-1701	CP Sil 19CB	PAS-1701	DB-1701	PE-1701	007-1701	Rtx-1701 MXT-1701	BP-10	SPB-1701
Trifluoropropyl	AT-210	—	—	DB-210	—	—	Rtx-200	—	—
50% Methyl– 50% phenyl	AT-50	—	HP-17 HP−50+	DB-17 DB-17 HT DB-608	PE-17	007-17	Rtx-50	—	SP-2250 SPB-50
65% Diphenyl- 35% methyl	—	TAP-CB	—	—	—	400 −65 TG	Rtx-65 Rtx-65 TG	—	—
50% Cyanopropyl methyl– 50% phenylmethyl	AT-225	CP Sil 43CB	HP-225	DB-225	PE-225	007-225	Rtx-225	—	—

Carbowax	AT-Wax	CP Wax 52 CB	HP-20 M InnoWax	DB-Wax	PE-CW	007-CW	Stabilwax MXT-Wax	BP-20	Supelcowax-10 Omegawax Carbowax PEG 20M
Carbowax (basic)	—	—	—	CAM	—	—	Stabilwax	—	Carbowax-Amine
Carbowax (acidic)	AT-1000 FFAP	CP Wax -58 CB	HP-FFAP	DB-FFAP OV-351	PE-FFAP	007-FFAP	Stabilwax-DA	BP-21	Nukol SP1000
90% Biscyanopropyl–	AT-	—	—	—	—	—	Rtx-2330	—	SP2330
10% phenylcyanopropyl	Silar						Rt-2330		SP2331 SP2380 SP2560
100% Biscyanopropyl	—	CP Sil 88	—	—	—	—	Rtx-2340		SP2340
				Specialty Phases					
EPA VOCs	AT-624	CP Sil 13 CB	—	DB-624	PE-502	007-624	Rtx-Volatiles	—	VOCOL
EPA Method 502.2	—	—	HP-VOC	DB-624 DB-VRX	PE-502	007-502	RTx-502.2	—	VOCOL
524.2									
EPA Method 504	—	—	—	DX-3	—	—	Rtx-Volatiles	—	—
EPA Method 608	AT-5	CP Sil 8	HP-5,PAS-5	DB-5	PE-2	007-2	Rtx-5	BP-5	SPB5
	AT-35	—	HP-608	—	PE-608	007-608	Rtx-35	—	SPB608
	AT-1701	CP Sil 19	PAS-1701	DB-1701	PE-1701	007-1701	Rtx-1701	BP-10	SPB1701
	AT-50	—	HP-50	DB-17 DB-608	PE-17	007-17	Rtx-50	—	SPB608
				Chiral Phases					
	Chirasil-Val (Alltech), Cyclodex-B (J&W), Rtx-βDEXm (Restek), Cyclex-B (SGE), B-DEX (Supelco)								
				PLOT Columns					
	Molesieve 5A (Chrompack), MoleSiev 5A (HP), GS-Molesiev (J&W), PLT-5A (Quadrex)								
	PoraPlot Q (Chrompack), GS-Q (J&W), Poraplot Q (HP)								
	GS-Alumina (J&W), Al_2O_3/KCL (Chrompack), Aluminum oxide/KCl (HP), Aluminum oxide/Na_2SO_4(HP)								

Source: Column designations obtained from 1994/95 Catalog of Chromatography Products, Bellefonte, PA.

fact that their viscosity is nearly independent of temperature (104). However, the introduction of phenyl and more polar functionalities on the polysiloxane backbone causes a decrease in viscosity of a polysiloxane at elevated temperatures, resulting in thermal instability. Four areas have enhanced the quality of stationary phases for capillary GC: (1) in situ free-radical crosslinking of stationary phases coated on fused silica, (2) the synthesis or commercial availability of a wide array of highly viscous gum phases, (3) the use of OH-terminated polysiloxanes, and (4) the use of polysilphenylene-siloxanes, sometimes referred to *polyarylene–siloxane* phases, which will be discussed later in this section.

3.11.2 Cross-Reference of Columns from Manufacturers

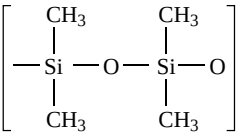
A cross-reference of capillary columns offered by the major column manufacturers (listed in alphabetical order) is presented in Table 3.20 and serves as a handy reference of stationary phases and their chemical composition when comparing columns and chromatographic methods. This author hastens to add that the information presented here is the best available at the time and, as stated earlier, not all-inclusive. Column manufacturers continue to add MS grade, polyarylene–polyphenylene–siloxane, and method-specific columns to their product line on a regular basis; the reader is urged to consult technical information available from vendors or on their Websites. Perusal of this table indicates several trends in the bewildering array of column designations, which in itself is testimony to the widespread use of capillary GC. Each manufacturer has its own alpha or numeric designation for is product line, such as HP (HP-), J&W (DB-), Restek (RT-), SGE (BD-), and Supelco (SPB-). In many instances, the numerical suffix corresponds to the numerical suffix of the appropriate OV (Ohio Valley) phase appearing in Table 3.8 and is representative of the percentage of the polar functional group or modifier in a given polysiloxane. For example, HP-5 is listed as being chemically similar to DB-5 in terms of its chromatographic properties, including its selectivity and retention characteristics. For a given polysiloxane, likewise, inference should not be drawn that the phases Rtx-50 and SP-2250 are identical, only that they are chemically similar and behave similarly under chromatographic conditions. Since each manufacturer has optimized column preparation for a specific stationary phase, column dimensions, and, in some cases, an intended application of the column, slight differences in chromatographic behavior are to be expected. A manufacturer considers the steps involved in column preparation to be proprietary information.

Two types of stationary phases are most popular: the polysiloxanes and polyethylene glycol phases. Both types of phases may be characterized as having the necessary high viscosity and the capability for crosslinking and/or chemical bonding with fused silica. One should note the presence of more recent additions to the capillary column family, namely, specialty columns designed for selected EPA methods, chiral separations, and gas–solid chromatographic separations. These specialty phases are considered in Section 3.11.6.

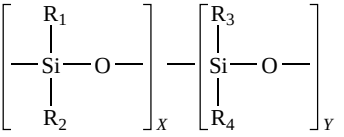
3.11.3 Polysiloxanes

Polysiloxanes are the most widely used stationary phases for packed- and capillary-column GC. They offer high solute diffusivities coupled with excellent chemical and thermal stabilities. The thorough review of polysiloxane phases by Haken (105) and the overview of stationary phases for capillary GC by Blomberg (106,107) are strongly recommended readings.

One measure of the polarity of a stationary phase is the cumulative value of its McReynolds constants, as discussed in Section 3.6.3. Because a variety of functional groups can be incorporated into the structure, polysiloxanes exhibit a wide range of polarities. Since many polysiloxanes are viscous gums and, as such, coat well on fused silica and can be crosslinked, they are ideally suited for capillary GC. The basic structure of 100% dimethylpolysiloxane can be illustrated as



Replacement of the methyl groups with another functionality enables polarity to be imparted to the polymer. The structure of substituted polysiloxanes in Tables 3.8 and 3.20 and Figure 3.6 can be depicted by the following general representation



where the R groups can be CH_3, phenyl, $CH_2CH_2CF_3$, or $CH_2CH_2CH_2CN$, and X and Y indicate the percentage of an aggregate in the overall polymeric stationary phase composition, as described in Figure 3.6. In the case of the phase, DB-1301 or one of its chemically equivalents (6% cyanopropylphenyl–94% dimethylpolysiloxane, $R_1 = CH_2CH_2CH_2CN$, R_2 = phenyl, R_3 and R_4 are methyl groups; and X and Y have the values of 6 and 94%, respectively. For phases equivalent to 50% phenyl–50% methylpolysiloxane, R_1 and R_2 are methyl groups, while R_3 and R_4 are aromatic rings; X and Y are each equal to 50%. Additional polysiloxanes of various polarities have been described in the literature having polar functionalities of 4-(methylsulfonylphenyl) (108) and polyhydroxysubstitution (109) as well as medium polarity phases having methoxy-terminated (110) and silanol-terminated silarylene/siloxane goups (111).

Phase selectivity, which also impacts resolution in a chromatographic separation, is governed by solute–stationary-phase interactions, such as dispersion, dipole, acid/base, and hydrogen bond donors/acceptors. A column containing a polar stationary phase can display greater retention for a solute having a given

polar functional group compared to other solutes of different functionality, while on a less polar stationary phase elution order may be reversed or altered to varying degrees under the same chromatographic conditions. Poole has investigated selectivity-equivalency of polydimethyldiphenylsiloxane stationary phases for capillary GC (112). Several illustrations of how elution order is affected by stationary phase selectivity are presented in Figures 3.38 and 3.39.

3.11.4 Polyethylene Glycol Phases

The most widely used non-silicon-containing stationary phases are the polyethylene glycols. They are commercially available in a wide range of molecular weights under several designations, such as Carbowax 20M and Superox-4. The general structure of a polyethylene glycol may be described as

$$HO–CH_2–CH_2–(–O–CH_2–CH_2–)_n–O–CH_2–CH_2–OH$$

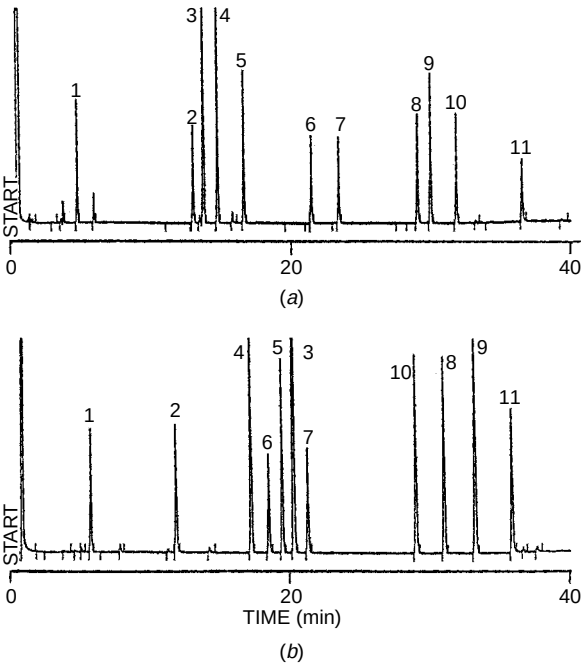


FIGURE 3.38 Chromatograms of the separation of nitrogen-containing compounds on 10-m × 0.53-mm-i.d. column with (a) CP Sil 19 CB and (b) CP Sil 8 CB as stationary phase. Column conditions: 50°C (3 min) at 6°C/min to 250°C. Det: FID, 24 cm/s He. Solutes: (1) nitropropane, (2) 2-nitro-2-methyl-1-propanol, (3) *n*-dodecane, (4) nitrobenzene, (5) *o*-nitrotoluene, (6) 2-nitro-2-methyl-1,3-propanediol, (7) 2-nitro-2-ethyl-1,3-propanediol, (8) *p*-nitroaniline, (9) *p*-nitrobenzyl alcohol, (10) *o*-nitrodiphenyl, and (11) 4-nitrophthalimide (Reference 146). The stationary phases, CP Sil 19 CB and Cp Sil 8 CB, are chemically similar to OV-1701 and OV-5, respectively. (E. F. Barry and R. Brophy, unpublished results.)

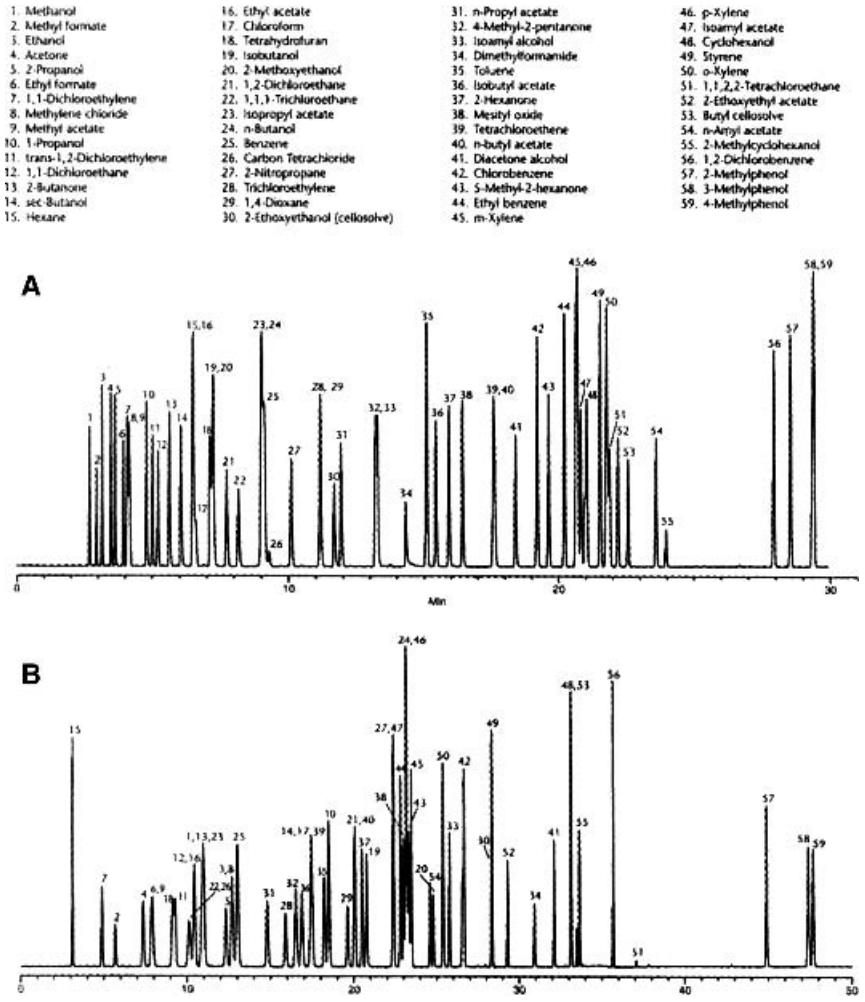


FIGURE 3.39 Chromatograms illustrating differences in selectivity for components of an industrial solvent mixture with (a) Equity-1 stationary phase; (b) SUPELCOWAX stationary phase. In each case column dimensions: 30-m × 0.32 mm-i.d., 1.0 μm; temperature conditions: 35°C (8 min) at 4°C/min to 130°C. Det: FID, 25 cm/s He, split injection (200:1). (Reprinted with permission of Supelco, Bellefonte, PA.)

The popularity of polyethylene glycols stems from their unique selectivity and high polarity as a liquid phase. Unfortunately, they do have some limitations. Characteristic of Carbowax 20M, for example, is its rather low upper temperature limit of approximately 225°C and a minimum operating temperature of 60°C. In addition, trace levels of oxygen and water have adverse effects on most liquid phases, but particularly so with Carbowax 20M, where they accelerate the degradation process of the phase. Verzele and co-workers have

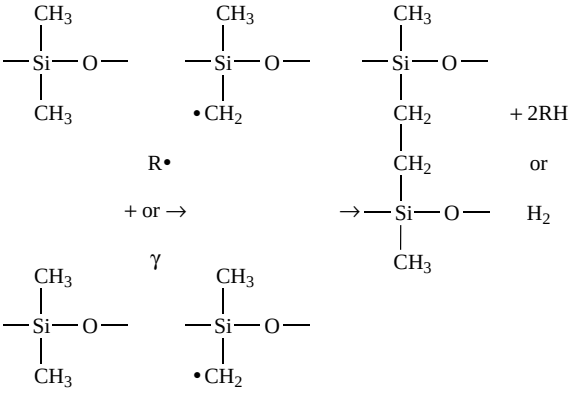
attempted to counteract these drawbacks by preparing a very high-molecular-weight polyethylene glycol, Superox-4 (113). Other successful attempts include free-radical crosslinking and bonding, which are discussed in the next section.

3.11.5 Crosslinked versus Chemically Bonded Phases

The practice of capillary GC has been enriched by the advances made in the immobilization of a thin film of a viscous stationary phase coated uniformly on the inner wall of fused-silica tubing. At present, two pathways are employed for the immobilization of a stationary phase: free-radical crosslinking and chemical bonding. By immobilizing a stationary phase by either approach, the film is stabilized and is not disrupted at elevated column temperatures or during temperature programming. Thus, less column bleed and higher operating temperatures can be expected with a phase of this nature, a consideration especially important in GCMS. A column containing an immobilized stationary phase is also recommended for on-column injection and large volume injectors/cool on-column inlets where large aliquots of solvent are injected without dissolution of the stationary phase. Likewise, a column having an immobilized phase can be backflushed to rinse contamination from the column without disturbing the stationary phase (Section 3.11.7).

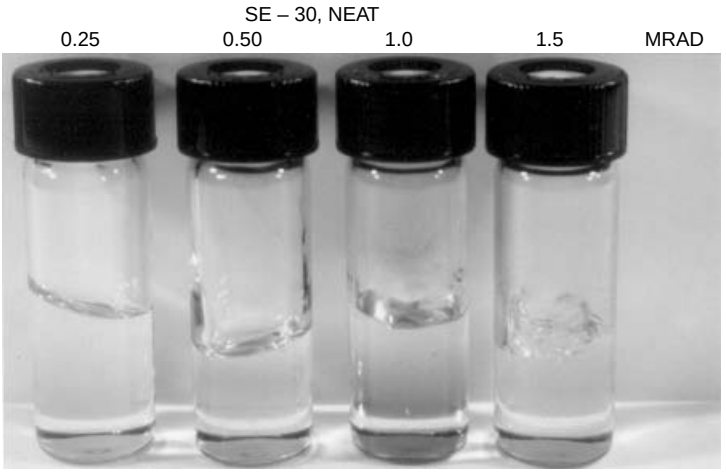
3.11.5.1 Crosslinking of a Stationary Phase

The ability of a polymer to cross-link is highly dependent on its structure. The overall effect of crosslinking is that the molecular weight of the polymer steadily increases with the degree of crosslinking, leading to branched chains until eventually a three-dimensional rigid network is formed. Since the resultant polymer is rigid, little opportunity exists for the polymer chains to slide past one another, thereby increasing the viscosity of the polymer. On treatment of a crosslinked polymer with solvent, the polymer does not dissolve, but rather a swollen gel remains behind after decantation of the solvent. Under the same conditions, an uncrosslinked polymer of the same structure would dissolve completely. In summary, the dimensional stability, viscosity, and solvent resistance of a polymer are increased as a result of crosslinking. The mechanism for crosslinking 100% dimethyl polysiloxane is described below where R• and γ (gamma radiation) are free-radical initiators:



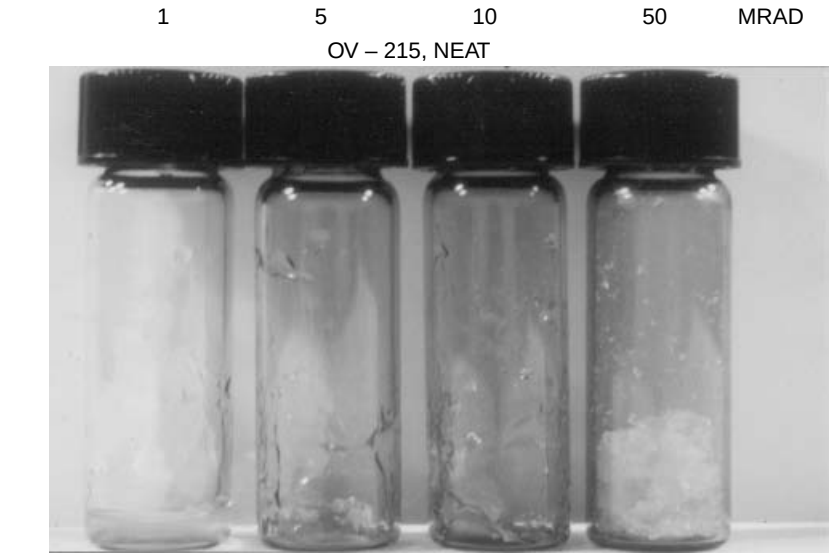
The sequence of photographs presented in Figure 3.40 permits a helpful visualization of the crosslinking process for several stationary phases, 100% dimethylpolysiloxane (SE-30), and trifluoropropylmethylpolysiloxane, as a function of increasing degree of crosslinking by ^{60}Co gamma radiation (114). In Figures 3.40c,d the conversion of DC200 (a silicone oil) and OV-101 (a polysiloxane fluid) to crosslinked gel versions as a function of radiation dosage is illustrated.

Madani et al. provided the first detailed description of capillary columns where polysiloxanes were immobilized by hydrolysis of dimethyl and diphenylchlorosilanes (115,116). Interest increased when Grob found that the formation of crosslinked polysiloxanes resulted in enhanced film stability (117). Blomberg et al. illustrated in situ synthesis of polysiloxanes with silicon tetrachloride as a precursor, followed by polysiloxane solution (118,119). Since then, various approaches for crosslinking have been investigated. These include chemical additives such as organic peroxides (120–128), azo compounds (82,129,130), ozone (131), and gamma radiation (77,132–135). Several different peroxides have been evaluated; dicumyl peroxide is the most popular. However, peroxides can generate polar decomposition products that remain in the immobilized film of stationary phase. Moreover, oxidation may also occur, which increases the polarity and decreases the thermal stability of a column. These adverse effects are eliminated with azo species as free radical initiators. Lee et al. have crosslinked a wide range of stationary phases, from nonpolar to polar, in their studies using azo-*tert*-butane

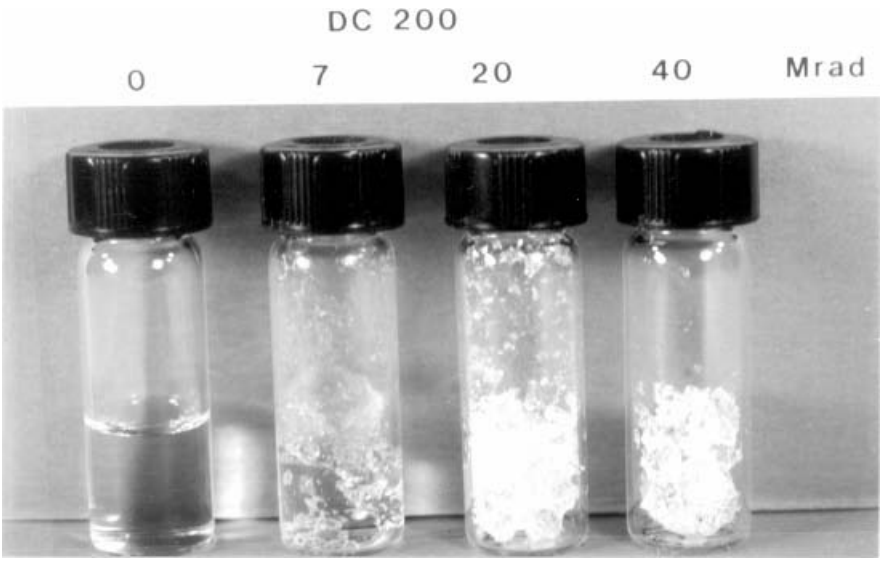


(*a*)

FIGURE 3.40 Effect of gamma radiation on degree of crosslinking of (a) SE-30 (polydimethylsiloxane; (b) OV-215 [trifluoropropylmethylpolysiloxane (Reference 133)]; (c) DC 200 (a silicone oil); (d) conversion of OV-101 (a polydimethylpolysiloxane fluid) to a gum similar to OV-1. [Parts (a) and (b) reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a Division of Preston Industries, Inc. and reprinted with permission of Preston Publications, Inc.]

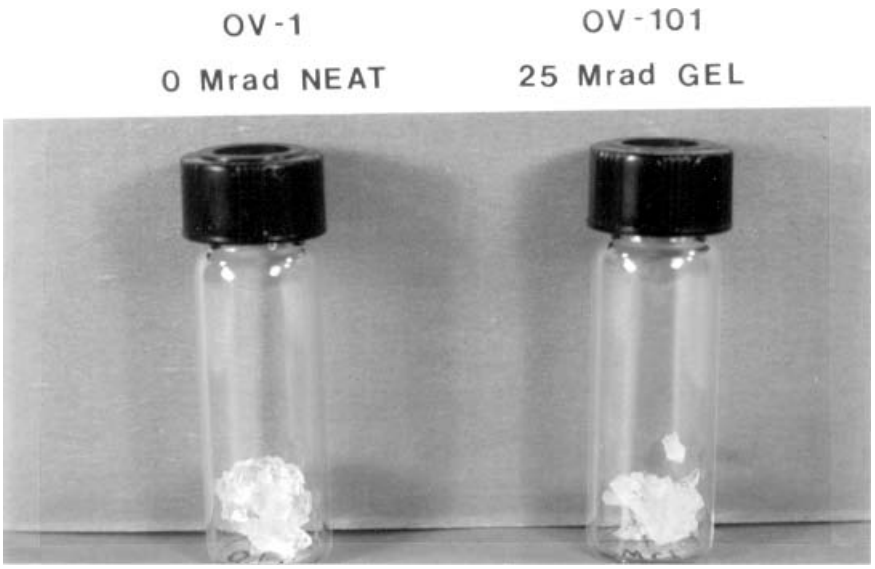


(*b*)



(*c*)

FIGURE 3.40 (*Continued*)



(*d*)

FIGURE 3.40 (*Continued*)

and other azo species (82,129,130). If an azo compound or a peroxide exists as a solid at room temperature, the agent is spiked directly into the solution of the stationary phase used for coating of the column. On the other hand, for free-radical initiators that are liquid at ambient temperature, the column is first coated with stationary phase, then saturated with vapors of the reagent (47,82).

Gamma radiation from a ^{60}Co source has also been used (77,114,132–135) as an effective technique for crosslinking polysiloxanes. In a comparative study of gamma radiation with peroxides, Schomburg et al. (77) noted that each approach immobilized polysiloxanes, but that the formation of polar decomposition products is avoided with radiation. Radiation offers the additional advantages of the crosslinking reaction occurring at room temperature, and columns can be tested both before and after immobilization of the stationary phase.

Not all polysiloxanes can be directly or readily crosslinked. The presence of methyl groups facilitates crosslinking. Consequently, the nonpolar siloxanes exhibit very high efficiencies and high thermal stability. However, as the population of methyl groups on a polysiloxane phase decreases and as these groups are replaced by phenyl or more polar functionalities, crosslinking of a polymer becomes more difficult. Incorporation of vinyl or tolyl groups into the synthesis of a polymer tailored for use as a stationary phase for capillary GC overcomes this problem. Lee (126,129) and Blomberg (127,128) have successfully synthesized and crosslinked stationary phases of high phenyl and high cyanopropyl content that also contain varying amounts of these free-radical initiators. Colloidal particles have also been utilized to stabilize cyanoalkyl stationary-phase films

for capillary GC (136). More recently, favorable thermal stability and column inertness were obtained by a binary crosslinking reagent, a mixture of dicumyl peroxide and tetra(methylvinyl)cyclotetrasiloxane (137).

Developments in the crosslinking of polyethylene glycols have been slower in forthcoming, although successes have been reported. Immobilization of this phase by the following procedures increases its thermal stability and its compatibility and tolerance for aqueous solutions. DeNijs and de Zeeuw (138) and Buijten et al. (139) immobilized a PEG in situ, the latter group with dicumyl peroxide and methyl(vinyl) cyclopentasiloxane as additives. Etler and Vigh (140) used a combination of gamma radiation with organic peroxides to achieve immobilization of this polymer, while Bystricky selected a 40% solution of dicumyl peroxide (141). George (7) and Hubball (142) have successfully crosslinked PEG using radiation; in Figure 3.41 an array of vials of Carbowax 20M after receiving various dosages of gamma radiation is pictured, indicating that crosslinking has been achieved (vial D). The chromatographic separation of a cologne in Figure 3.42 was generated on a capillary column containing Carbowax 20M crosslinked by gamma radiation and indicates acceptable thermal stability to 280°C. Horka and colleagues described a procedure for crosslinking Carbowax 20M with pluriisocyanate reagents (143). Thermally bondable PEGs and polyethyleneimines have been popular phases and yield chromatographic selectivity similar to those of the traditional PEGs. Despite these efforts, the upper temperature limit of PEG columns generally remains below 300°C.

3.11.5.2 Chemical Bonding

Since the early nineties, column manufactures have devoted extensive resources to acquiring technology for the development of chemically bonding a stationary-phase

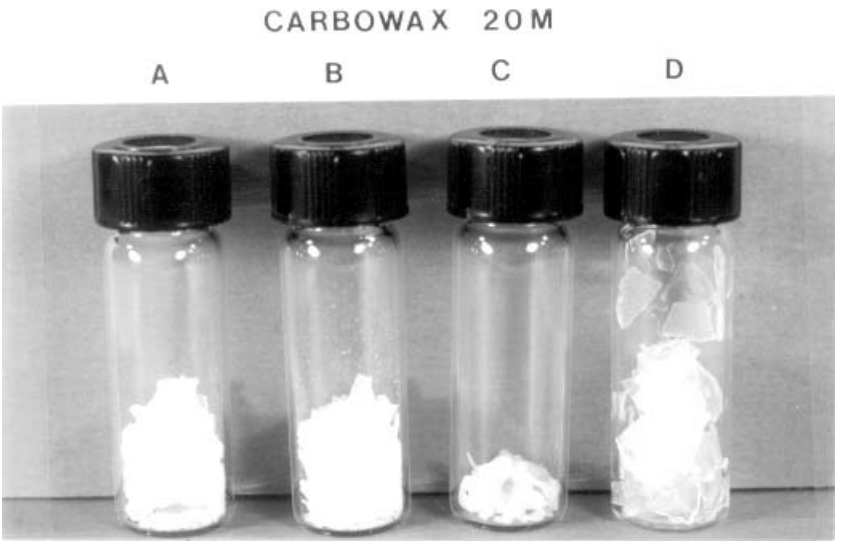


FIGURE 3.41 Effect of gamma radiation on degree of crosslinking of Carbowax 20M.

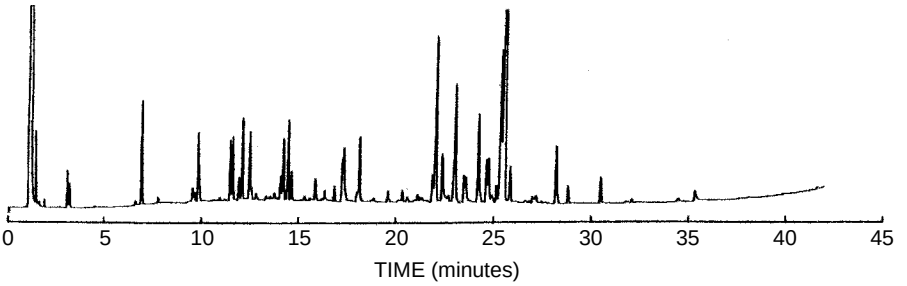


FIGURE 3.42 Chromatogram illustrating the separation of a cologne sample on a capillary column (15 m × 0.32-mm-i.d., 0.25-μm film) containing Carbowax 20M crosslinked by gamma radiation; column conditions: 40°C (2 min) at 6°C/min to 280°C. Det: FID, 25 cm/s He.

film to the inner wall of a fused-silica capillary. As the term suggests, an actual chemical bond is formed between fused silica and the stationary phase. The foundation of this procedure was first reported by Lipsky and McMurray (144) in their investigation of hydroxy-terminated polymethylsilicones and was later refined by the work of Blum et al. (145–149), who employed OH-terminated phases for the preparation of inert, high-temperature stationary phases of varying polarities. The performance of hydroxy-terminated phases has also been evaluated by Schmid and Mueller (150) and Welsch and Teichmann (151). Other published studies include the behavior of hydroxyl phases of high cyanopropyl content by David et al. (152) and trifluoropropylmethylpolysiloxane phases by Aichholz (153).

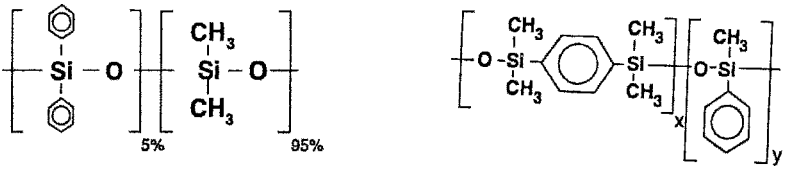
In the chemical bonding approach to stationary phase immobilization, a capillary column is coated in the conventional fashion with an OH-terminated polysiloxane and then temperature-programmed to an elevated temperature, during which time a condensation reaction occurs between the surface silanols residing on the fused-silica surface and those of the phase. It is important to note here that both deactivation and coating are accomplished in a single-step process and result in the formation of a Si–O–Si bond more thermally stabile than the Si–C–C–Si bond created via crosslinking. Crosslinking of the stationary phase is not a necessary requirement. However, if a stationary phase contains a vinyl group (or another free-radical initiator), crosslinking can occur simultaneously. Phases that cannot be crosslinked during the bonding process can be crosslinked afterward with an azo compound, for example. Grob, after observing the increased inertness and thermostability of OH-terminated phases, commented that they might reflect a "revolution in column technology" (154).

Since the mid-1980s, immobilization of polysiloxanes and polyethylene glycols has no longer been a subject of rapid advancements reported in the literature; a procedural blend of polymeric synthesis, crosslinking, and/or chemically bonding is utilized by column manufacturers today, as the fixation of these stationary phases via crosslinking and/or chemical bonding for capillary GC is now a well-defined and a matured technology. A capillary column containing such a stationary phase is the resultant of elegant pioneering efforts of people such as

M. L. Lee, L. Blomberg, K. Grob and his family members, G. Schomburg and their colleagues, and many, many others too numerous to mention here. Attention has now shifted to such areas as silphenylene(arylene) phases for GCMS, MS-grade phases, stationary-phase selectivity tuned or optimized for specific applications, multidimensional chromatographic techniques, and immobilization of chiral stationary phases, which are discussed in Section 3.11.6.

3.11.5.3 MS-Grade Phases versus Polysilarylene or Polysilphenylene Phases

Many column manufacturers offer what has become known as "MS columns," namely, columns that generate lower bleed than do regular or conventional polysiloxane equivalents. Lower bleed is highly desirable in GCMS analyses because complications such as bleed ions, misidentifications of compounds and errors in quantitation, to name few, are avoided. A MS-grade column may contain (1) a higher-molecular-weight polymer obtained by a fractional procedure of the corresponding starting stationary-phase version; (2) a polymer resulting from a crosslinking of a higher-molecular-weight fraction of the starting polymer; (3) a "crossbonded" polymer resulting from a condensation reaction of a hydroxyl-terminated polymer of either a conventional polysiloxane or a higher-molecular-weight fraction with fused silica where crosslinking may or may not have occurred, as discussed in the previous section; or (4) the stationary phase may be a polysilarylene-siloxane, often referred to as an *arylene phase*, or as a polysilphenyl-siloxane, often referred to as a *phenylene phase*. These latter phases are inherently more thermally stable because of the presence of aromatic rings in the polymer chain as depicted in Figure 3.43 and represent an upgrade in thermal performance over the corresponding polysiloxane counterpart, but one may also notice slight differences in selectivity due to different chemistry employed both in the deactivation procedure and also in a possible synthesis of the phase itself. Thus, cross-reference column charts should be carefully examined when comparing columns offered by various vendors when selecting a capillary column for an application requiring low column bleed level.



5% Phenyl Methylpolysiloxane **5% Phenyl Polysilphenylene-siloxane**

FIGURE 3.43 Structures of 5% phenyl–95% methylpolysiloxane and 5% phenylpolysilphenylene–siloxane as stationary phase.

3.11.5.4 Solgel Stationary Phases

Sol-gel is basically a synthetic glass with ceramic-like properties. The processing consists of hydrolysis and condensation of a metal alkoxide, specifically, tetraethoxysilane to form a glassy material at room temperature. Further modification of this material with a polymer (stationary phase) is used to prepare phases for capillary columns; there has been keen interest in this process (155–160). The final sol-gel product retains the properties of the polymer as well as properties of the sol-gel component. The sol-gel material is able to covalently bond to fused silica, yielding a strong bond, which means better thermal stability and less column bleed. In addition, the molecular weight of the stationary phase is stabilized via end-capping chemistry, providing protection from degradation and potential further condensation. At the present time, two sol-gel phases have been developed, a SolGel-1 ms derived from 100% dimethylpolysiloxane and the other, SOLGEL-WAX, which has PEG in the matrix. A cross-sectional view of a SOLGEL-WAX column along with a corresponding view of a conventionally coated capillary is presented in Figure 3.44; an application of a separation with this type of column appears in Figure 3.45.

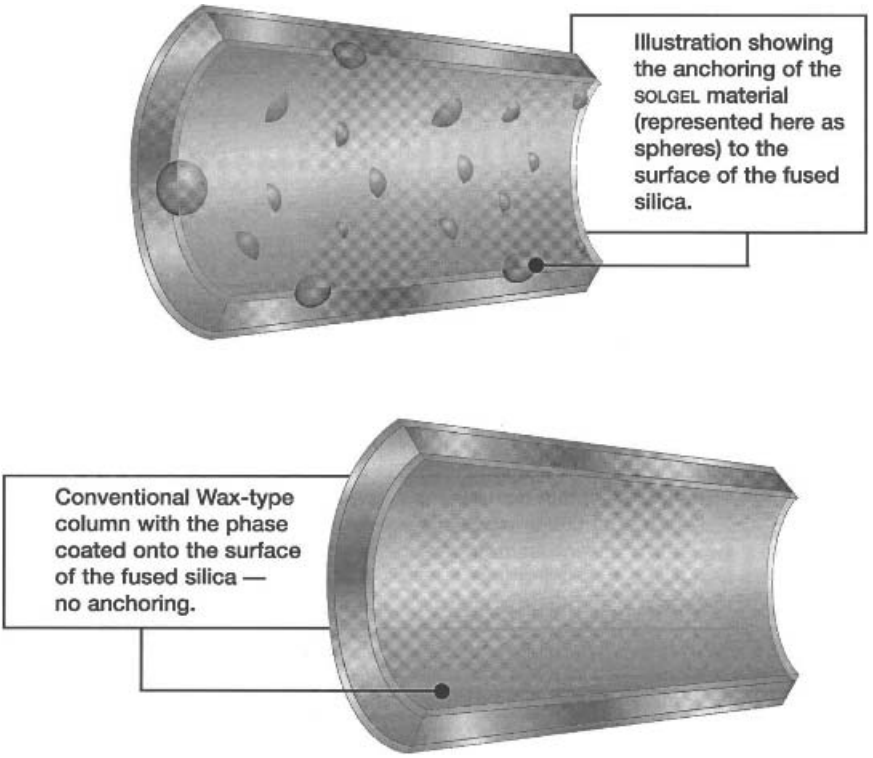


Illustration showing the anchoring of the SOLGEL material (represented here as spheres) to the surface of the fused silica.
Conventional Wax-type column with the phase coated onto the surface of the fused silica — no anchoring.

FIGURE 3.44 Comparison of cross section of a SOLGEL-WAX Column with a conventional wax-type column. (Reproduced with permission of SGE International Pty.)

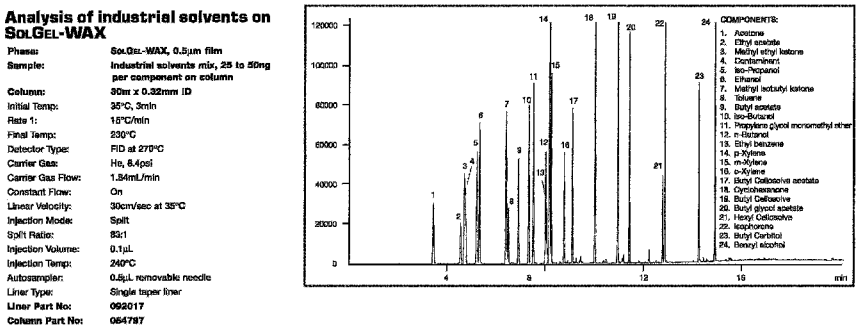


FIGURE 3.45 Separation of industrial solvents on a SOLGEL-WAX column. Column: 30 m × 0.32-mm-i.d., 0.5-μm film: Temperature conditions: 35°C (3 min) at 15°C/min to 230°C. Det: FID, 1.84 mL/min, 30 cm/s He, split injection (83–1) 240°C (Reproduced with permission of SGE International Pty.)

3.11.5.5 Phenylpolycarborane-Siloxane Phases

This classification of phases can be traced back to the previous use of a carborane-type phase termed Dexsil, which was widely utilized as a stationary phase in packed columns for high-temperature separations because of its excellent thermal stability. The carborane network has been incorporated into the backbone of phenylpolysiloxane phases having either a 5% or 8% phenyl content, providing unique selectivity for selected applications. In Figure 3.46, the structure of this modified polysiloxane is presented. A capillary column containing this stationary phase exhibits high selectivity for difficult-to-separate Aroclor 1242 congeners because of the carborane functionality in the polysiloxane polymer. An example of this is shown in Figure 3.47, where the carborane phase interacts preferentially with ortho-substituted PCB congeners, namely, congeners 28 and 31.

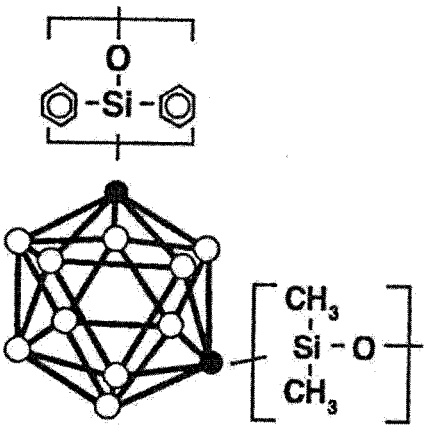


FIGURE 3.46 Structure of a phenylpolycarborane–siloxane stationary phase.

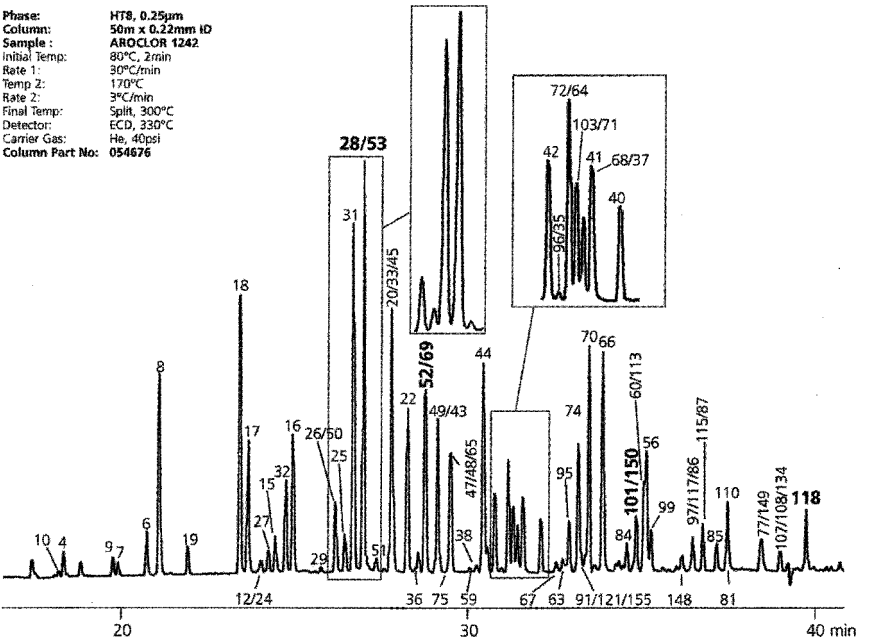


FIGURE 3.47 Chromatographic separation of Aroclor 1242 on a capillary column containing HT8 phenyl polycarborane–siloxane as stationary phase; column: 50 m × 0.22-mm-i.d., 0.25-μm film; temperature conditions: 80°C (2 min) at 30°C/min to 170°C, then 3°C/min to 300°C. Det: ECD, 1.84 mL/min, 40 psi He, split injection. (Reproduced with permission of SGE International Pty.)

3.11.6 Specialty Columns

3.11.6.1 EPA Methods

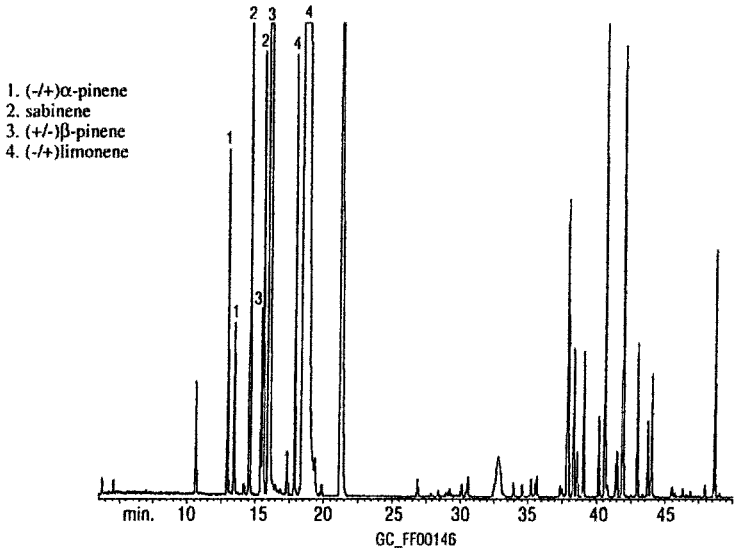
Column manufacturers have responded to the increasing analytical and environmental demands for capillary columns for use in EPA 500 Series, EPA 600 Series, and EPA 8000 Series methods. To simplify the column selection for a given EPA method by a user, a column is so designated, for instance, the alpha or numeric tradename of the manufacturer—the EPA method number, as shown in Table 3.20. These columns have been configured in length, inner diameter, film thickness, and stationary-phase composition for optimized separation of the targeted compounds under the chromatographic conditions specified in a particular method. Another factor relating to column dimensions considered by manufacturers is the compatibility of thicker-film columns with methods that stipulate purge-and-trap sampling by eliminating the need for cryogenic solute focusing prior to chromatographic separation.

3.11.6.2 Chiral Stationary Phases

Capillary columns having a chiral stationary phase are used for the separation of optically active isomers or enantiomers, namely, species that have the same

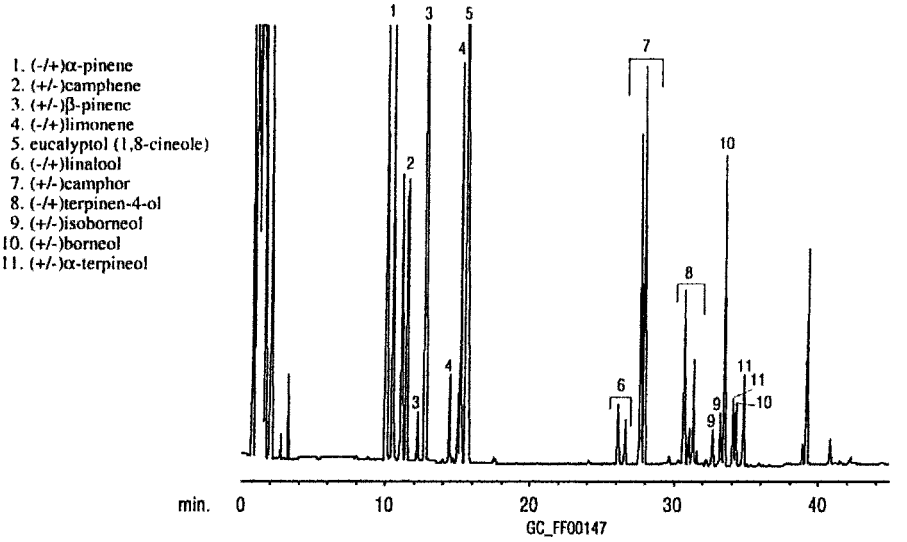
physical and chemical properties with the exception of the direction in which they rotate plane-polarized light. Enantiomers may also have different biological activity and, therefore, enantiomeric separations are important in the food, flavor, and pharmaceutical areas. A chiral stationary phase can recognize differences in the optical activities of solutes to varying extent whereas common stationary phases do not. In Figure 3.48 chromatograms illustrating the separation of enantiomers present in lemon and rosemary oils are found.

Most of the earlier chiral phases have limited thermal stability. By chemically bonding a chiral stationary phase to a polysiloxane, the upper temperature limit can be extended. Chirasil-Val is perhaps the most famous stationary phase in this category. However, more recent work has employed β-cyclodextrin as the key chiral recognition component in stationary phases. The mechanism of separation (or enantiomeric selectivity) is based on the formation of solute–β-cyclodextrin complexes occurring in the barrel-shaped opening of the cyclodextrin and can be manipulated by varying the size of the opening of the cyclodextrin ring as well as by the alkyl substitution pattern on the ring. Although cyclodextrins can be used as a stationary phase (161,162), the current practice is to either place them in solution with a polysiloxane (163) or immobilize them by bonding to a polysiloxane such as a cyanopropyl–dimethylpolysiloxane (164). The chromatographic principles underlying chiral separations by GC have been reviewed by Hinshaw (165).



(*a*)

FIGURE 3.48 Chiral analysis of (a) lemon oil and (b) rosemary oil. Column: 30 m × 0.32-mm-i.d., 0.25-μm film, Rt-βDEXsm. Temperature conditions: 40°C (1 min) at 2°C/min to 200°C (hold 3 min). Det: FID, 80 cm/s He, split injection. (Used with permission of the Restek Corp.)



(*b*)

FIGURE 3.48 (*Continued*)

3.11.6.3 Gas–Solid Adsorption Capillary Columns (PLOT Columns)

These columns are also referred to as PLOT or *porous-layer open tubular columns*. A PLOT column consists of fused-silica capillary tubing in which a layer of an adsorbent lines the inner wall in place of a liquid phase. An early use of a PLOT column was reported by de Nijs (166), who prepared a fused-silica column coated with submicron particles of aluminum oxide for the analysis of light hydrocarbons. De Zeeuw et al. (167) subsequently prepared PLOT columns with a 10–30-mm layer of a porous polymer of Porapak Q (styrene–divinylbenzene) by in situ polymerization of a coating solution. Because of improved column technology, adsorbent-type stationary-phase particles can now be bonded very effectively to the inner surface texture of fused silica, minimizing the need for particulate traps and the possibility of column particles accumulating in a FID jet. Several separations on PLOT are displayed in Figures 3.49 and 3.50. The selection of adsorbents currently available in a PLOT column format includes aluminum oxide/KCl, aluminum oxide/sodium sulfate, molecular sieves, alumina, graphitized carbon, and porous polymers. These columns are intended to be direct replacements for a packed column containing the same adsorbent and feature faster regeneration of the adsorbent.

3.11.7 Capillary Column Care and First Aid

3.11.7.1 Ferrule Materials and Fittings

Ferrules for capillary columns are usually fabricated from graphite and Vespel/graphite composites. Graphite ferrules are easy to use and have a higher

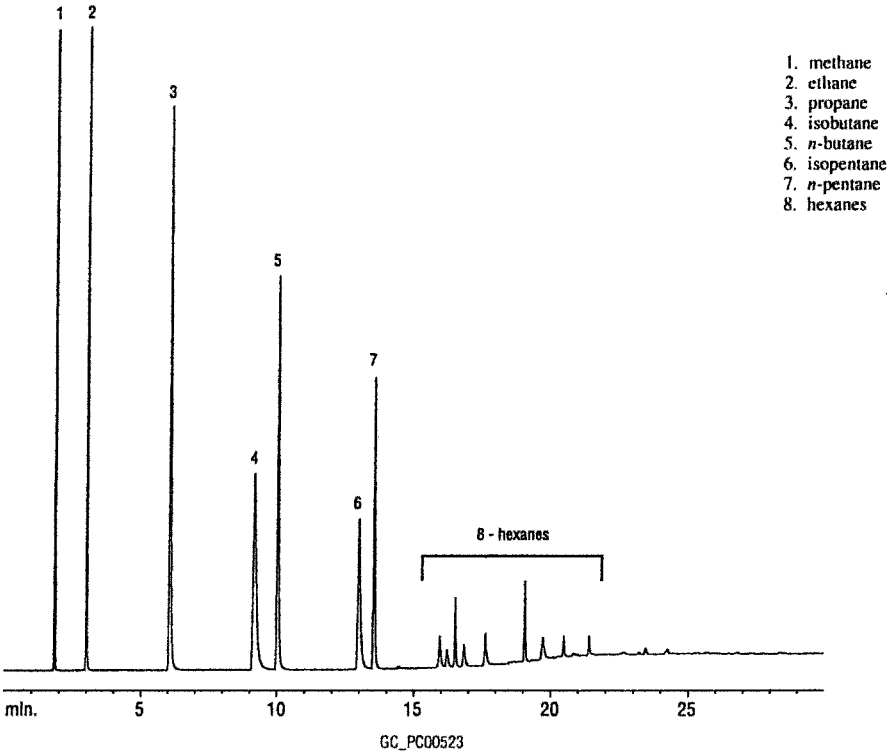
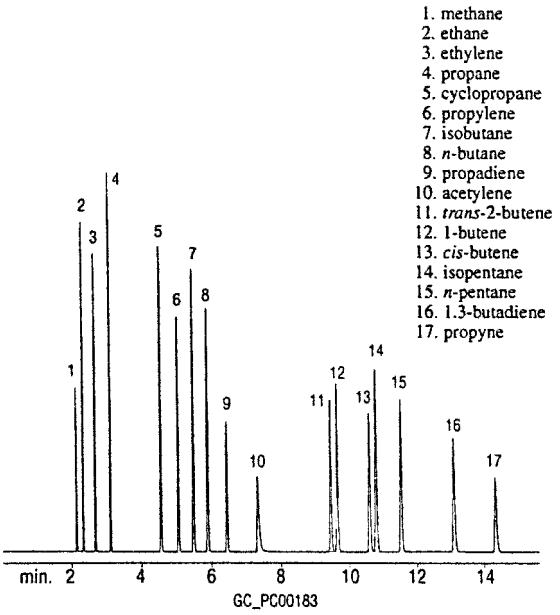


FIGURE 3.49 Separation of hydrocarbon gases on Rt-QPLOT column. Column: 30-m × 0.32 mm-i.d., Rt-QPLOT; temperature conditions: 40°C at 10°C/min to 240°C (hold 10 min). Det: FID, 18 psi He, 35 cm/s, split injection. (Used with permission of the Restek Corp.)

temperature limit but are softer, more easily deformed and are not recommended for GCMS systems. Vespel/Graphite composite ferrules are harder and thus do not deform as easily and, therefore, are recommended for GCMS systems. The characteristics of these materials are presented in Table 3.12. An alternate ferrule technology has been emerged with the SilTite metal ferrules. Graphite and graphite/vespel composites are made from different materials to the metal nuts so the connection components expand and contract at different rates as the column oven temperature changes. With a metal ferrule system, since the components are made from the same material, the components expand and contract at the same rate during changes in oven temperatures. SilTite metal ferrules have an inherently higher temperature limit and being metallic, the risk of MS contamination is eliminated. This connection arrangement is described in Figure 3.51.

It is important to select the proper ferrule inner diameter (i.d.) to be compatible with the outer diameter (o.d.) of the capillary column, or a carrier-gas leak will result after installation. Ferrules having an i.d. of 0.4 mm are recommended for 0.25-mm-i.d. columns, 0.5-mm-i.d. ferrules are recommended for



50m. 0.53mm ID Rt-Alumina™ PLOT (cat. # 19701)
100µL gas-tight syringe split injection hydrocarbon mix. 1000ppm

Oven temp.:	40°C to 120°C @ 5°C/min. (hold 5 min.)
Inj. & det. temp.:	200°C
Carrier gas:	helium
Linear velocity:	37.5cm/sec. set @ 80°C (5.0mL/min.)
Split flow:	60mL/min.
FID sensitivity:	1.28×10^{-10} AFS

FIGURE 3.50 Separation of refinery gases on Rt-alumina PLOT column. Column: 50 m × 0.53 mm-i.d., Rt-QPLOT. Temperature conditions: 5°C at 10°C/min to 120°C (hold 5 min). Det: FID, He 37 cm/s, split injection, FID. (Used with permission of the Restek Corp.)

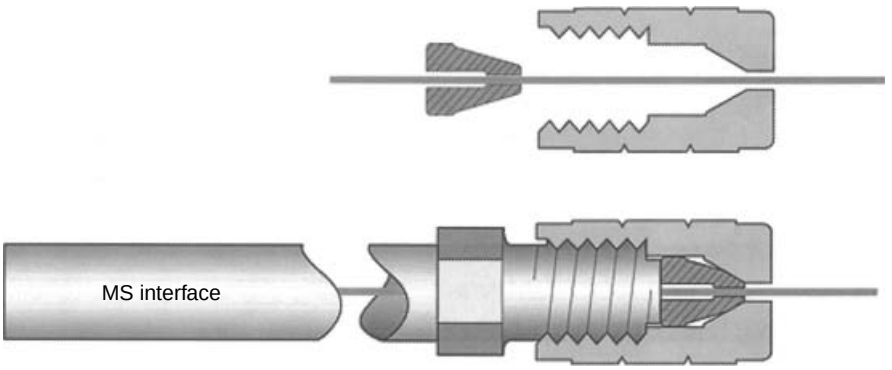


FIGURE 3.51 Illustration of an all-metal SilTite connection (reproduced with permission of SGE International Pty.)

0.32-mm-i.d. columns, and 0.8-mm-i.d. ferrules are recommended for 0.53-mm-i.d. capillary columns.

Outlined below are guidelines for the preparation of a capillary column for installation.

1. Slide the retaining fitting or nut over the end of a new column and then the ferrule, and position them at least 6 in. away from the column end. It will be necessary to cut several inches from the end of the column because ferrule particles may have entered the column and can cause tailing and adverse adsorptive effects.
2. With a scoring tool, gently scribe the surface of the column several inches away from the end. While holding the column on each side of the scoring point, break the end at the scoring point at a slight downward angle. Any loose chips of fused silica or polyimide will fall away and will not enter the column, as may happen if it is broken in a completely horizontally configuration. This procedure eliminates the possibility of chips of fused silica or polyimide from residing in the end of the column. Alternatively, an excellent cut can be made with a ceramic wafer (Figure 3.52).
3. Examine the end of the column with a $10-20\times$ magnifier or an inexpensive light microscope. The importance of a properly made cut cannot be overstated. An improperly cut column, as illustrated in Figure 3.53, where a series of problematic scenarios are clearly evident, can generate active sites and may cause peak tailing, peak splitting, or solute adsorption.

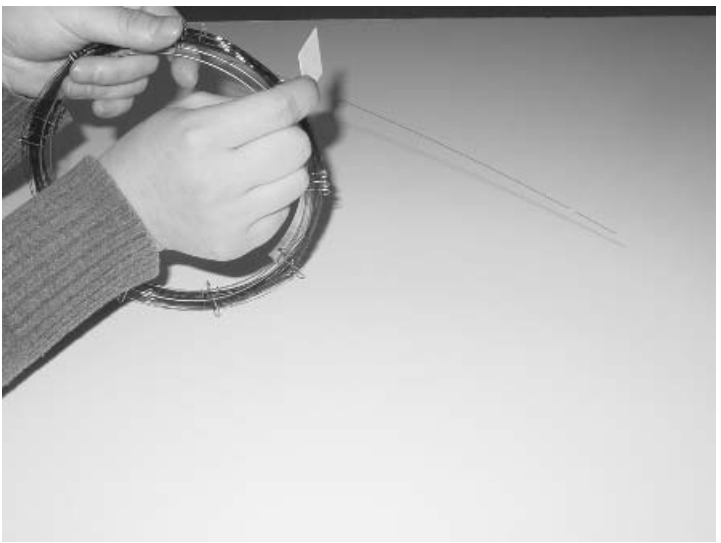
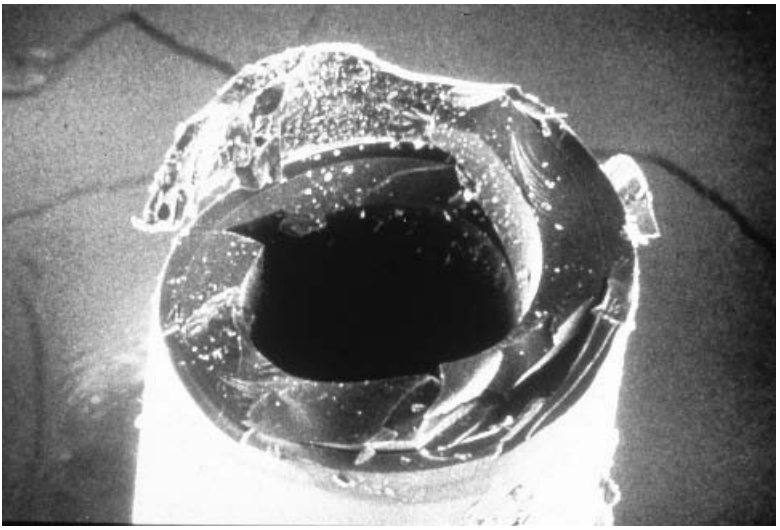
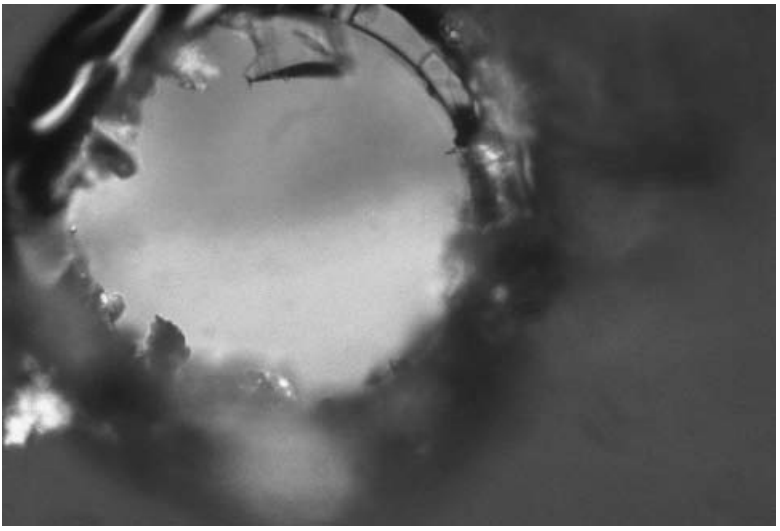


FIGURE 3.52 Termination of an end of a fused-silica capillary column with a ceramic wafer.

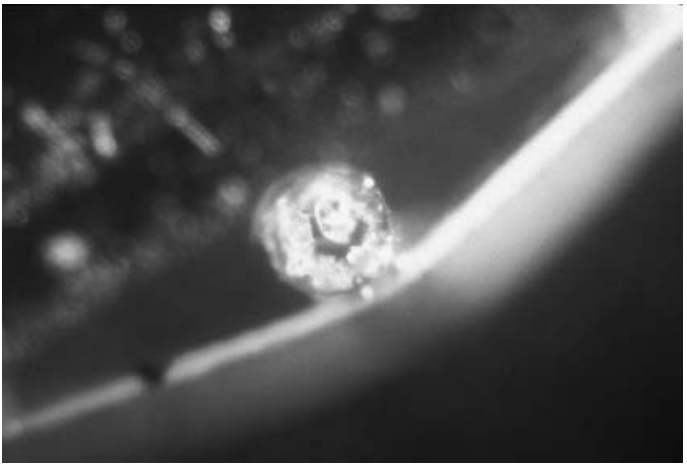


(*a*)

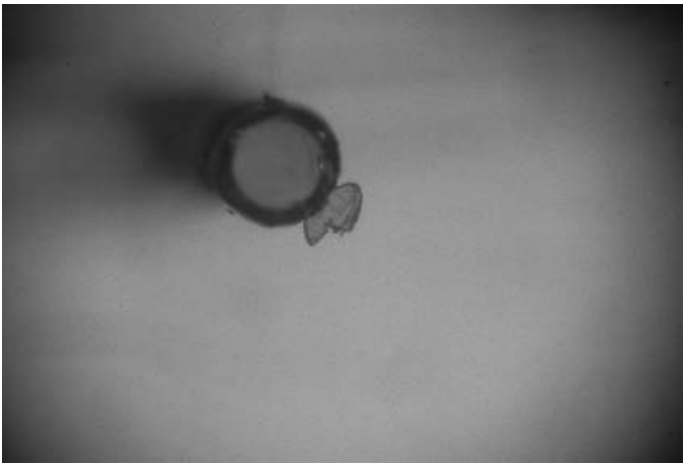


(*b*)

FIGURE 3.53 Scenarios that can occur in cutting an end of a capillary column: (a) jagged protrusion; (b) jagged inner diameter; (c) particles of fused silica accumulated at inlet; (d) piece of polyimide attached to column end; (e) an acceptable cut (courtesy of Agilent Technologies).



(*c*)



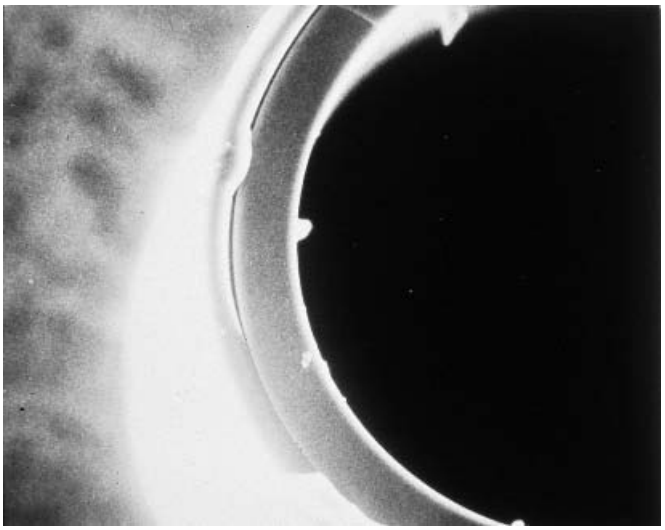
(*d*)

FIGURE 3.53 (*Continued*)

4. A supply of spare ferrules and related tools (Figures 3.54 and 3.55) are convenient to have in the laboratory when removal or changing columns is required. Ferrules, ferrule-handling accessories, toolkits, and other handy gadgets are available from many column manufacturers.

3.11.7.2 Column Installation

Define the injector and detector ends of the column. Align the end to be inserted into the injector with a ruler, and mark the recommended distance of insertion as specified in the instrument manual with typewriter correction fluid. Then slide



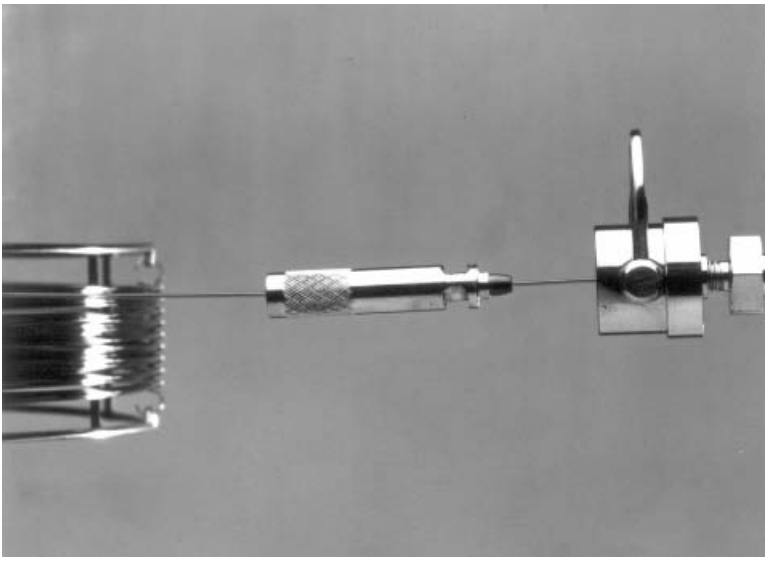
(*e*)

FIGURE 3.53 (*Continued*)

FIGURE 3.54 Photograph of a capillary-column ferrule kit containing an assortment of ferrules and a pin vise drill with bits for drilling or enlarging the bore of ferrules (reprinted with permission of Supelco, Bellefonte, PA).

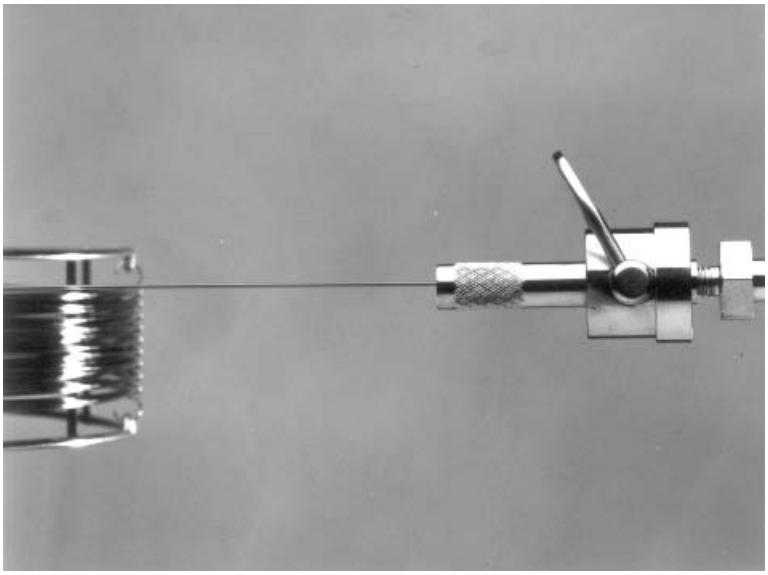
the ferrule and nut closer to the point of application of the correction fluid and mount the column cage on the hanger in the column oven. Alleviate any stress, sharp bends, or contact with sharp objects along the ends of the column. Insert the measured end into the injector and tighten the fitting. If the column is to be conditioned, leave the detector end of the column disconnected; otherwise, insert this end into the jet tip of the FID at the specified recommended distance, usually 2 mm down from the top of the jet. In Figure 3.56 a photograph of the

FIGURE 3.55 Photograph of a capillary-column toolkit containing tweezers, needle files, scoring tool, pin vise drill kit, flow calculator, pocket mirror, miniflashlight, labels, septum puller, stainless-steel ruler, and pipe cleaners (reprinted with permission of Supelco, Bellefonte, PA).



(*a*)

FIGURE 3.56 Illustration of the quick-connect fitting for installation of capillary columns: (a) internal sealing mechanism and (b) sealing mechanism inserted into fitting and locked into place (photographs courtesy of the Quadrex Corp.)



(*b*)

FIGURE 3.56 (*Continued*)

quick-connect fitting is shown. This device facilitates column installation in most gas chromatographs without the use of wrenches and extends ferrule lifetime.

3.11.7.3 Column Conditioning

Conditioning of a capillary column removes residual volatiles from the column. There are three essential rules for conditioning a capillary column, the first two of which also apply to the conditioning of a packed column (Section 3.7.5):

1. Carrier-gas flow must be maintained at all times when the column temperature is above ambient temperature, and there should be no gas leaks.
2. Do not exceed the maximum allowable temperature limit of the stationary phase, or permanent damage to the column can result.
3. As opposed to the conditioning of a packed column, overnight conditioning of a capillary column is usually unnecessary. Instead, purge the column with normal carrier-gas flow for 30 min at room temperature, then temperature-program the column oven at 4°C/min to a temperature 20°C above the anticipated highest temperature at which the column will be subjected without exceeding the maximum allowable temperature limit. Usually after a column has been maintained at this elevated temperature for several hours, a steady baseline is obtained and the column is ready for analyses to be conducted. Use of high-purity carrier gas, a leak-free chromatographic system, and following the guidance of Chapter 10 will greatly extend the lifetime

of any column, packed or capillary. Other details pertaining to conditioning and column care can be found in Section 3.7.5.

3.11.7.4 Column Bleed

Column bleed is a term used to describe the rise in baseline during a blank temperature programming run and is the inevitable consequence of increasing vapor pressure and thermal degradation of a polymer with an increase in column temperature as well as that due to the accumulation of nonvolatiles in the column, as shown in Figure 3.57. One should expect some degree of bleeding with every column; some phases just generate more bleed than others. Always try to select a stationary phase of high thermal stability. For example, a nonpolar phase bleeds less than a polar phase because the former typically has a higher temperature limit and thus is more thermally stable. Moreover, in comparing capillary columns of different dimensions, the level of bleed will increase with increasing amount of stationary phase in the column. Therefore, longer and wider-diameter columns yield more bleed than do shorter or narrower columns. Likewise, column bleed increases with increasing film thickness of the stationary phase and with increasing column length. Reducing film thickness and using a shorter or narrower column will result in less bleeding.

The rate of temperature programming or ramp rate can influence the bleed profile from a column. As the rate of temperature programming increases, column bleed also increases. Finally, the more sensitive element-specific detectors (e.g., an ECD or NPD) will generate a more pronounced bleed profile if the stationary phase contains a heteroatom or functional group (–CN or –F) to which a detector responds in a sensitive fashion.

3.11.7.5 Retention Gap and Guard Columns

A 0.5–5.0 m length of deactivated fused-silica tubing installed between the injector and analytical column is often referred to as a retention gap or guard column (Figure 3.58). The term, retention gap, is used to describe this segment for on-column injection where the condensed solvent resides after injection, but both solvent and solutes are not retained once vaporization occurs via temperature programming. As a guard column, this short length of deactivated tubing preserves the lifetime of an analytical column by collecting nonvolatile components and particulate matter in dirty samples that would otherwise accumulate at the inlet of the analytical column. As such, its latter role in capillary GC parallels the function of the guard column in HPLC. A guard column is considered to be a consumable item, requiring replacement from time to time, usually when the detector response of active analytes begins to decrease substantially. It eliminates the need for the repetitive removal of small sections at the inlet end of an analytical column with the buildup of contamination.

Proper implementation of the connection between the guard and analytical columns is essential for the preservation of the chromatographic integrity of the system. The generation of active sites within the fitting can cause adsorptive losses and peak tailing. Commercially available fittings for this purpose include

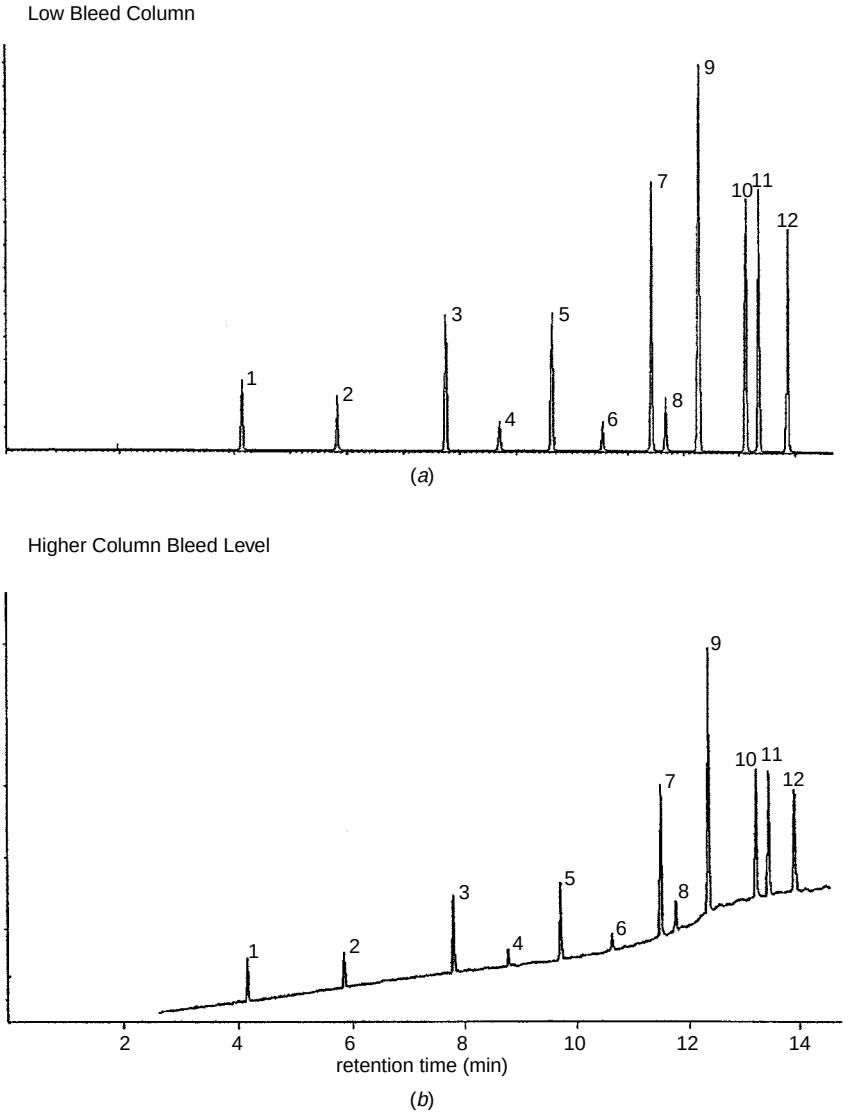
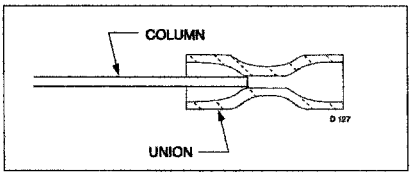


FIGURE 3.57 Comparison of column bleed from (a) a relatively new capillary column with a bleed profile from (b) a frequently used column of identical dimensions and film thickness under the same gas chromatographic conditions.

the metal butt connector of low dead volume, press-tight connectors, and a capillary Vu-Union. An illustration of the primary and secondary sealing mechanisms in the capillary Vu-Union is shown in Figure 3.59, where the two column ends are positioned into ferrules located inside a deactivated tapered glass insert. This type of connector combines the benefits of a low dead volume connection with



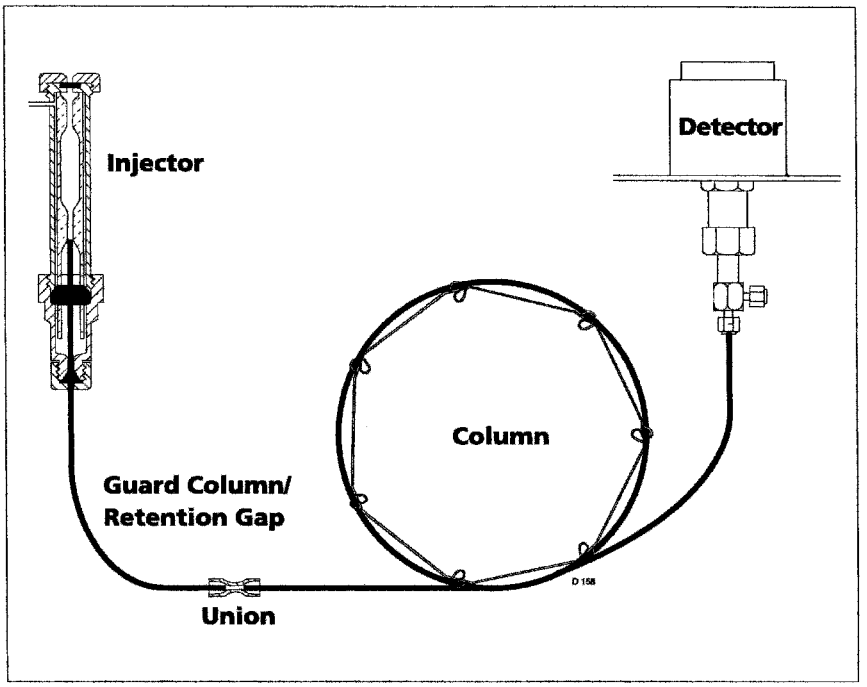


FIGURE 3.58 Schematic diagram of a guard column/retention gap (illustration courtesy of Walter Jennings, consultant).

the sturdiness of a ferrule seal. Furthermore, the glass window permits visual confirmation of the connection.

3.11.7.6 Column Fatigue and Regeneration

Deterioration in column performance can occur by contamination of the column with the accumulation of nonvolatiles and particulate matter, usually in the injector liner and column inlet. Column contamination is manifested by adsorption and peak tailing of active analytes, excessively high column bleed levels, and changes in the retention characteristics of the column. Rejuvenation of the column can be attempted by several paths. First, remove one or two meters of column from the inlet end. If the column still exhibits poor chromatographic performance, try turning the column around and reconditioning it overnight disconnected from the

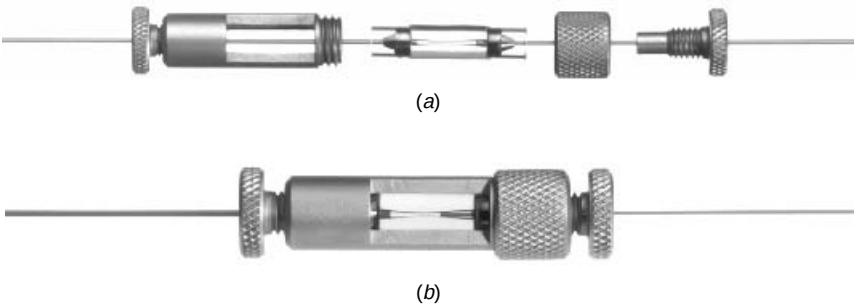


FIGURE 3.59 Photographs of a (a) disassembled capillary/micro-bore Vu-Union showing the primary and secondary sealing mechanisms and (b) assembled (photographs courtesy of the Restek Corp.)

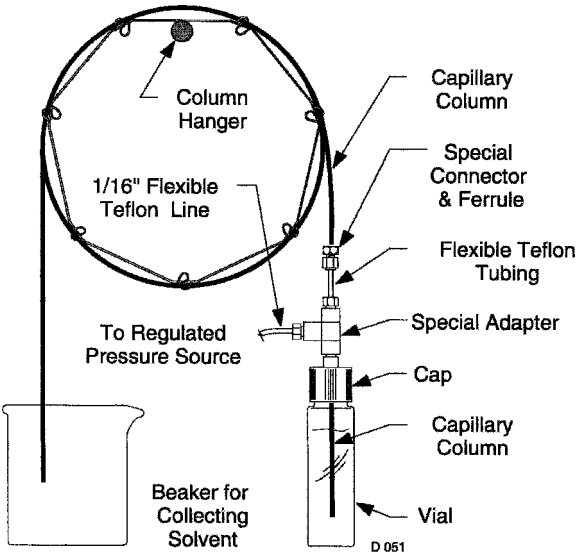


FIGURE 3.60 Schematic diagram of a solvent rinse kit (illustration courtesy of Walter Jennings, consultant).

detector. A third approach is solvent rinsing, an extreme measure that should be attempted only with crosslinked or chemically bonded phases. Solvent-rinse kits, such as the one schematically described in Figure 3.60, are commercially available and enable a column to be backrinsed of contamination by slowly introducing 10–30 mL of an appropriate solvent into the detector end of the column by nitrogen gas pressure. The results of this procedure appears in Figure 3.61. This approach is worthwhile for heavily contaminated columns, but in all cases the recommendations for rinsing outlined by the column manufacturer should be followed.

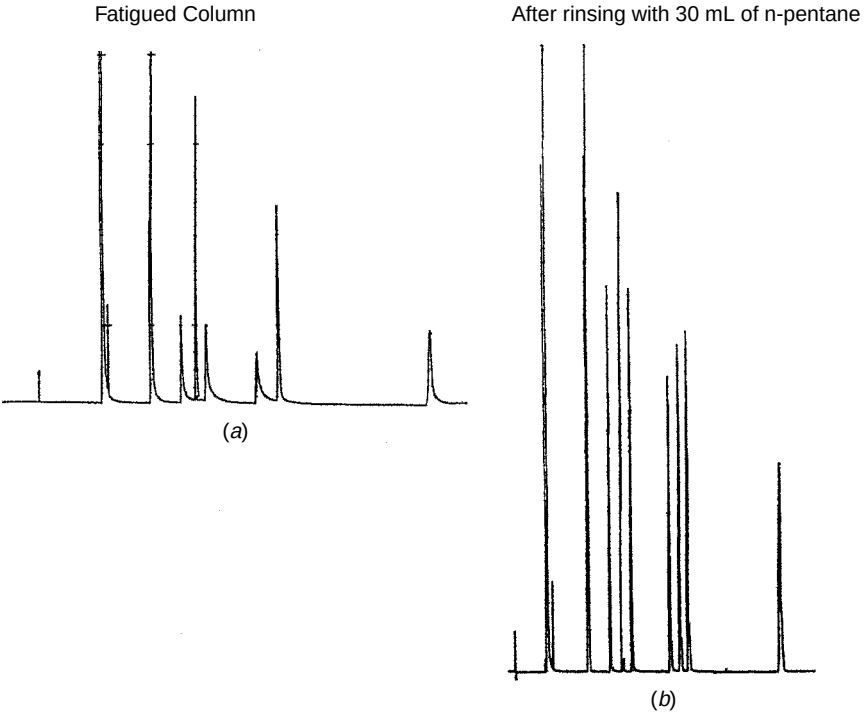


FIGURE 3.61 Illustration of regeneration of a capillary column by solvent rinsing with *n*-pentane.

3.11.8 Applications

Numerous applications of capillary separations appear throughout the remaining chapters of this book and provide the reader with an appreciation for the vast scope and power of capillary GC. A condensed summary of some general application areas and a corresponding suggested stationary phase is presented in Table 3.21. This summary is obviously intended not to be all-inclusive, but rather to serve as a starting point for column selection.

PART 4 COLUMN OVEN TEMPERATURE CONTROL

3.12 THERMAL PERFORMANCE VARIABLES AND ELECTRONIC CONSIDERATIONS

Gas chromatographic columns are installed in a column oven where the temperature must be accurately and precisely controlled, because column temperature has a pronounced influence on retention time. Any fluctuation in column temperature

TABLE 3.21 Recommended Stationary Phases for Selected Applications

Stationary-Phase Polysiloxane	Application
100% methyl	Alkaloids, amines, drugs, FAME, hydrocarbons, petroleum products, phenols, ethanol, essential oils solvents, PCBs, simulated distillation, waxes, general purposes
5% phenyl–95% dimethyl	Alcohols, alkaloids, aromatic hydrocarbons, drugs, FAMEs, flavors, fuels, halogenates, herbicides, pesticides, petroleum products, solvents, waxes, general purposes
50% methyl–50% phenyl	Alcohols, drugs, herbicides, pesticides, phenols, steroids, antidepressants, sugars
14% cyanopropylphenyl–86% dimethyl	Alcohols, pesticides, herbicides, aroclors, PAHs, phenols, steroids, alcohol acetates, drugs fragrances, pesticides
50% Cyanopropylmethyl–50% phenyl	Carbohydrates, FAME
Trifluoropropyl	Drugs, environmental samples, ketones, nitroaromatics
Polyethylene glycol	Alcohols, aromatics, aldehydes, essential oils, glycols, pharmaceuticals, flavors, fragrances, FAME, amines, acids

will yield an impact on the measurement of retention data and retention indices. Present oven geometries and electronic temperature control components are capable of thermostatting a column oven to $\pm 0.1^{\circ}C$.

A column oven must satisfy several additional requirements. A column oven should be thermally insulated from heated injector and detector components, a requirement that becomes more demanding as the selected column oven temperature approaches ambient temperature. Ideally, the temperature of a column oven should remain constant and independent of environmental changes in the laboratory and any line voltage fluctuations. Versatility in the operating temperature capability is also necessary to achieve column temperatures ranging from subambient temperature to elevated temperatures above $400^{\circ}C$ (for separations with metal-clad capillary columns). With more recent advances in adsorbents and PLOT columns, the need for cryogenic cooling of a column oven for the subambient separations of permanent gases and light hydrocarbons is no longer required, but has been replaced by the need of cryogenic capability for solute focusing purposes with on-column injection and auxiliary sampling techniques, such as thermal desorption and purge and trap.

Current gas chromatographic oven design is also a product of the age of miniaturization. Early column ovens were relatively large in volume (up to 3500 $in.^3$) to

accommodate U-shaped glass columns for biomedical and preparative separations. Thermal gradients were common in these huge, vertically configured rectangular ovens. On the other hand, the typically smaller oven geometry of today (i.e., $30 \times 27 \times 15$ cm or $\sim$12 L) can comfortably accommodate two capillary columns, a packed and a capillary column, or two packed columns. Forced-air convection is the most popular type of gas chromatographic oven, because it provides a uniform temperature in the column oven. Modern oven designs also permit fast cooldown rates after temperature programming, an important consideration because it governs sample throughput in a laboratory.

In modern gas chromatographs the temperature controller of a column oven is a microprocessor incorporated into a feedback loop, allowing both temperature programming ramp profiles and isothermal heating to be accomplished accurately and reproducibly. Under microprocessor control, a flap or door movement permits the blending of the proper amount of ambient lab air with oven air in the control of oven temperature. In addition, a cryogenic valve can be opened by a microprocessor for delivery of carbon dioxide or liquid nitrogen in the column oven.

3.13 ADVANTAGES OF TEMPERATURE PROGRAMMING OVER ISOTHERMAL OPERATION

Isothermal operation of a chromatographic column has a number of drawbacks, as illustrated in the scenario depicted for the separation of lime oil in Figure 3.62. If the selected isothermal column temperature is too low, the early-eluting peaks will

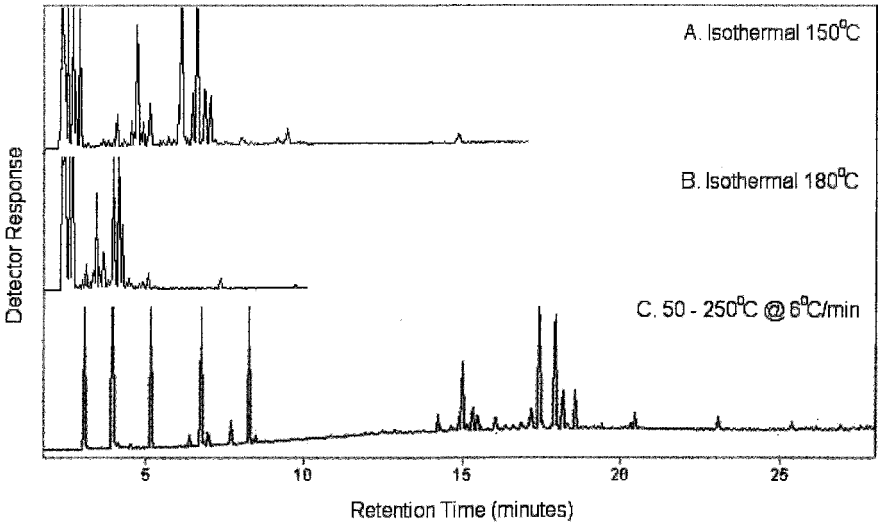


FIGURE 3.62 Gas chromatographic version of the general elution problem with a separation of lime oil. (P. Wilasuwan and E. F. Barry, unpublished results.)

be closely spaced, while the more strongly retained components will be broad and low-lying (Figure 3.62a). These strongly retained components can be more quickly eluted by selecting a higher isothermal temperature, which will also improve their detectability (Figure 3.62b). However, in doing so, more rapid coelution of components, peaks too closely spaced, and an overall loss in resolution result in the beginning of a chromatogram. This situation, which prevails in all practiced versions of elution chromatography, is often called the "general elution problem"; it is solved in GC by temperature programming, where the column oven temperature is gradually increased at a linear rate during an analysis (Figure 3.62c).

Temperature programming offers several attractive features. One can expect a reduced time of analysis and improved overall detectability of components (peaks are sharper and have nearly equal bandwidths throughout the chromatogram). In the case of unknown samples or samples of high complexity, high-boiling components, which might not elute or be detected under isothermal conditions, can exhibit a more favorable retention time. Temperature programming also helps "clean out" a column of remnant high-boiling species from previous injections. The interested reader is urged to consult the classic book *Programmed Temperature Gas Chromatography* by Harris and Habgood (168) for a detailed treatment of the subject.

3.14 OVEN TEMPERATURE PROFILES FOR PROGRAMMED-TEMPERATURE GC

Three basic types of temperature-programming profiles are used in GC: ballistic, linear, and multilinear. *Ballistic programming* occurs when an oven maintained at a given isothermal temperature is rapidly changed to a much higher isothermal temperature (Figure 3.63a) and is sometimes used for fast conditioning of a gas–solid chromatographic column after it has been unused for a period of time. More commonly, programming of this type is incorporated into peripheral

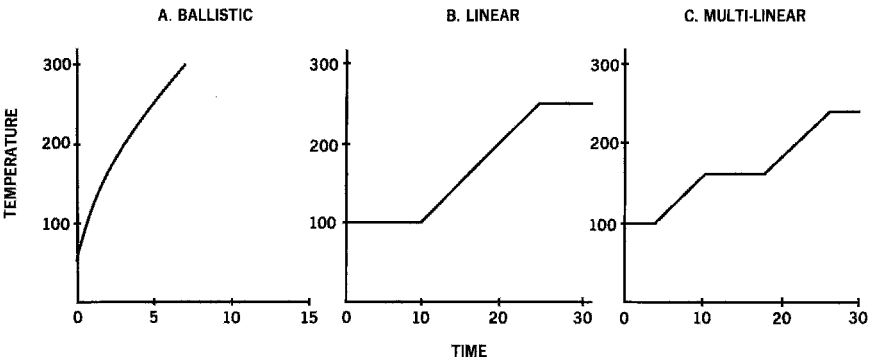


FIGURE 3.63 Types of temperature programming: (a) ballistic temperature programming; (b) linear temperature programming; and (c) multilinear temperature programming.

sampling methods to quickly drive off solutes from an adsorbent. An example is the purge-and-trap procedure for the determination of volatiles in aqueous solution, where collected solutes are thermally desorbed by ballistic programming (and also by rapid controlled linear temperature programming) from a silica gel/charcoal/Tenax trap so that they migrate as a narrow zone to the inlet of a capillary column where they are focused. A ballistic ramp may also be used with cryogenic solute focusing to elevate the column temperature quickly to above ambient temperature. However, a chromatographic column maintained at an elevated temperature, then ballistically programmed, can suffer severe damage due to disruption of the stationary phase film caused by this thermal shock.

The most widely used temperature program is the linear profile, as described in Figure 3.63b. Here the run begins at a low initial temperature, which may be maintained for a certain number of minutes (an isothermal hold), after which the column oven temperature is raised at a linear rate to the selected final temperature where it can also be maintained for a specific time interval. The initial temperature and hold period are usually determined from a scouting run made while noting elution temperatures; proper selection of the initial conditions will permit separation of the low boilers in the separation, while the final temperature chosen should be sufficient for the elution of the more strongly retained components in the sample (keeping in mind the upper temperature limit of the stationary phase). Multilinear profiles (Figure 3.63c) may be employed in some instances to fine-tune or enhance the resolution in a separation, but are more commonly used in conjunction with on-column injection. In this injection mode, a low column temperature is maintained during sample introduction into the retention gap, then initiation of the first and usually faster ramp rate induces the

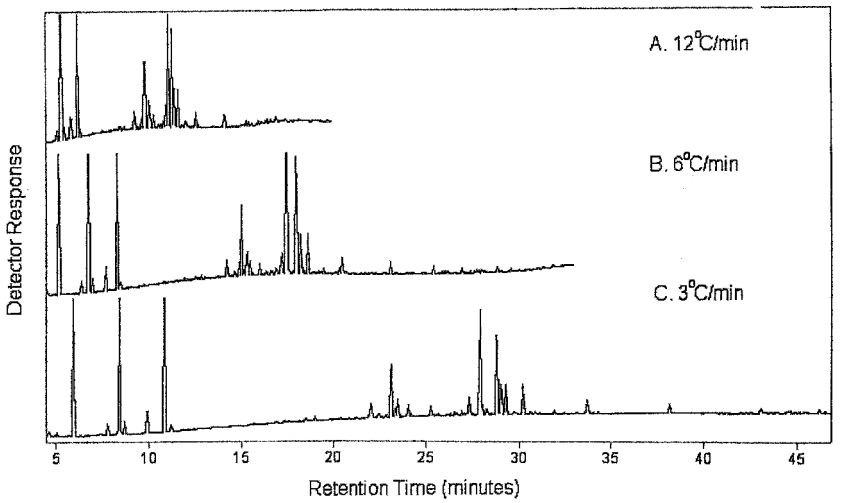


FIGURE 3.64 Effect of rate of temperature programming on resolution and analysis time; column: 30 m × 0.25-mm-i.d. DB-1 (0.25-μm film); split injection (100−1), 27 cm/s He. Det: FID (P. Wilasuwan and E. F. Barry, unpublished results).

solvent and components to start moving in the analytical column and the final ramp is implemented for elution of the components.

The ramp rate governs the tradeoff between analysis time and resolution. The compromise between resolution and time of analysis is contrasted in Figure 3.64 for parallel capillary column separations of lemon oil generated at three different programming rates. Relying on experience and intuition in establishing optimum column temperature conditions can be time-consuming and inefficient. An alternative route is the use of computer simulation for method development; this approach is discussed in much further detail in the next chapter (98–102).

3.15 CAPILLARY CAGE DESIGN

A capillary column is placed on a cage after it is prepared by a column manufacturer. A cage serves several purposes. Since the ends of flexible fused-silica capillary tubing are inherently straight, the column must be coiled and retained securely on the cage. To alleviate stress on coiled fused silica, the diameter of the cage must be compatible with the inner diameter on the column. In other words, the column cannot be coiled too tightly, or fracture of the tubing can occur. Megabore columns are coiled on 8-in. cages, whereas 0.10–0.32-mm-i.d. columns are wound on 5–7-in. frames.

The cage also confines the column such that it will not flop around in the column oven under the influence of forced-air convection currents. Before a column cage is mounted on the hanging bracket in the oven, the column should be examined to see if the coils are evenly spaced or distributed around the width of the cage with minimum overlap, a pertinent consideration for uniform heating of the column. It is also important that ends of the column extending from the cage for connection to the injector and detector do not contact any metallic parts of the oven where abrasion can erode the protective outer layer of polyimide on the column surface.

3.16 SUBAMBIENT OVEN TEMPERATURE CONTROL

Most gas chromatographs have the capability to operate the column oven at subambient temperatures. An accessory kit is available for either liquid nitrogen ($-99^{\circ}C$) or carbon dioxide ($-40^{\circ}C$) as a coolant and includes a cryogenic valve that is microprocessor controlled. The valve opens and closes, depending on the demand for coolant. In the open position, coolant is sprayed into the oven, where it chills the oven down with assistance from forced-air convection.

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CHAPTER FOUR

Optimization of Separations and Computer Assistance

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Optimization of gas chromatographic separations requires careful attention to a number of important variables and their interactions. In this chapter we will approach gas chromatographic optimization from the top down. First we will consider major options that have profound effects on a given separation, and which limit subsequent choices for many of the column variables, both physical—length, inner diameter, stationary phase—and parametric—temperature and flow or velocity. Then we will examine in more detail the questions of how to

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produce a desired change in a separation in order to approach an optimization goal. Finally, we will discuss a number of programmatic approaches to optimization solutions.

The ensuing discussion of gas chromatographic optimization primarily addresses goal-oriented modification of column dimensions and operating conditions. Column selection, particularly of the stationary phase, has been addressed in Chapter 3. Here we focus on the effects of changing the column operating conditions and the column dimensions.

This chapter focuses almost exclusively on capillary columns, which choice is not intended to dismiss the usefulness of packed column, but rather to reflect the preponderance of capillary columns in laboratories as well as the greater operational and dimensional parameter ranges they cover. According to a 2002 survey of gas chromatography users [1], nearly all respondents employed capillary gas chromatographic columns in their labs, while less than half reported packed-column use. A greater percentage used capillary columns in all the application areas addressed by the survey as well. Clearly, capillary columns are the preeminent choice today.

4.1 WHY OPTIMIZE?

Chromatographers optimize separations in order to achieve specific goals such as higher speed of analysis or improved peak-to-peak resolution. An increasing daily sample load, for example, can provide the impetus for higher speed of analysis when the purchase of additional instrumentation is a less desirable alternative. Problems with unexpected peaks or column degradation may be good reasons to improve peak resolution. Optimized separations can produce more efficient laboratory equipment utilization, higher sample throughput, better accuracy and repeatability, and longer column lifetimes, and can reduce detector maintenance.

Optimization of chromatographic separations can be complex. Not only are there a relatively large number of variables to consider, but also each variable interacts with others in significant ways. Increasing the average carrier gas linear velocity in an open tubular (capillary) column may produce more rapid separations, but at the same time the column efficiency may be compromised and critical pair peak resolution may drop below a minimum desired level. The situation can become even more complex when performing temperature-programmed elution—changing the linear velocity at the beginning or during separation may cause some peak retentions to shift relative to each other or even cause two peaks to reverse their elution order.

The range and scope of optimization variables also must be examined in the light of instrumental limitations and requirements. Choosing a very narrow-bore capillary column because it can deliver high resolution at high speeds does not guarantee that available instrumentation can produce the necessary thermal and pressure ranges. And likewise, some concomitant advanced techniques such as high-speed injection may lie outside an instrument's scope. Thus, past a certain

point optimization may become less desirable or even impractical as it invokes additional expense and knowledge acquisition that chromatographers are unwilling or unable to expend.

Successful optimization requires a level of expertise. Some simple optimization tasks, such as tuning up a temperature program or trimming column length, may be performed within limits by most practicing chromatographers. Other optimization goals that involve complex samples or extensive modifications, however, may be beyond even relatively experienced lab personnel. Lacking a resident guru-level gas chromatographer or the funds for a consultant, many labs choose to purchase the expertise in the form of computer optimization software. Ranging from relatively simple window diagramming to more involved thermodynamic prediction models, such programs are valuable tools in the modern chromatography laboratory. But even the most elaborate optimization program will fail to produce accurate results and predictions if the information fed to it is incorrect or inaccurate, or if the instrumentation to which the optimization is applied is not capable of imposing the required conditions on the selected chromatographic column or delivering the requisite detector response.

Although highly desirable, separations optimization often is not possible within the structured laboratory environments of many regulated organizations. A pharmaceutical quality control (QC) lab, for example, operates under strict controls that permit only the use of extensively validated methodology. Making any changes suggested by optimization procedures breaks the chain of validation, and the modified methodology must be re-validated. The added time and expense plus required personnel training and possibly new instrumentation diminish the attractiveness of optimization. In these and similar labs, new analytical challenges may provide the impetus for separations optimization.

4.2 BASIC CHOICES

The initial choices of column and supporting instrumentation have a profound influence on the possibilities for, and ultimate results of, separation optimization. Manipulation of column parameters—stationary phase, inner diameter, length, and film thickness—gives chromatographers control over column efficiency, resolution, and speed of analysis. The limits of inlet pressure, sampling systems, and detectors imposed by available instrumentation and methodology establish practical boundaries for the column variables and resulting chromatograms. The choice of stationary phase determines first and foremost, for a given sample, how much column efficiency a separation will require to meet minimum resolution levels. How rapidly that separation is generated is more a function of column dimension and elution condition choices.

When presented with a large number of closely related peaks, or with an unknown separation, chromatographers will err on the side of high resolution to ensure that peaks are not coeluted. For a well-characterized separation, however, optimization for speed or resolution may be attractive. In situations where peaks

are well separated with excess resolution, chromatographers may increase the speed of analysis by utilizing shorter columns, higher carrier-gas linear velocities, or larger column internal diameters. On the other hand, when one or more peak pairs are marginally resolved, longer and/or narrower columns will deliver higher plate numbers and increased resolution, but the analyst may have to sacrifice speed of analysis to do so. Small changes in temperature and pressure conditions may provide a needed resolution boost and allow the chromatographer to make do with the column at hand. An alternative solution may lie in choosing a different stationary phase that better separates the peaks interest.

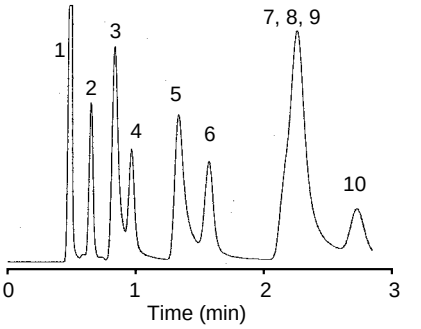
4.2.1 Packed versus Capillary Columns

One fundamental choice is the selection of a packed or capillary column. Packed columns generally tolerate misuse better than do capillary columns; they certainly are much less expensive, and they require simpler instrumentation to enable them to deliver all of their potential resolution. However, they do not cover as wide application or operating condition ranges, nor do they deliver the potential for speed of analysis with capillary columns. In certain areas, such as instrumentation for operation outside a controlled lab environment, the higher robustness of packed columns often makes them the primary choice. Some applications, such as many gas analyses, are frequently better served by packed columns with application-specific phases and supports, such as porous polymers or molecular sieves, although great strides have been made toward suitable capillary columns since the early 1990s.

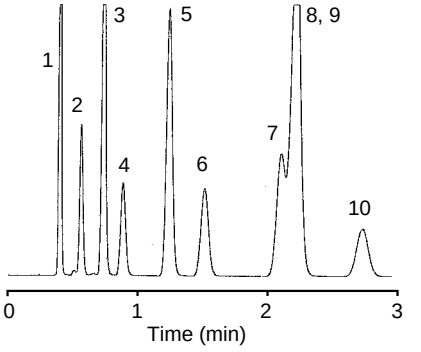
Leaving behind these packed-column-friendly application areas, a simple example will help demonstrate why capillary columns abound in gas chromatographic laboratories and at the same time begin our discussion of optimization. Figure 4.1a shows a chromatogram of a test mixture that contains both aromatic and nonpolar solutes on a 2-m long $\times$ 2-mm-i.d. nonpolar packed column at close to optimum carrier-gas flow. Note that the hydrocarbon peaks (peaks 1, 2, 4, 6, and 10) are symmetric, while the aromatic solutes exhibit significant peak tailing, due primarily to residual adsorptive sites on the column-packing material.

In Figure 4.1b, a 25-m-long $\times$ 0.53-mm-i.d. capillary column has been substituted for the packed column. All other parameters, including the column temperature, flowrate, injection size, and injection technique were not changed. The retention time of the last peak is essentially the same, but the overall quality of the chromatogram is greatly improved. The aromatic peaks no longer exhibit tailing, due now to a lack of adsorptive sites in the capillary column, and all the peaks have somewhat better resolution. It is this kind of immediate improvement obtained with capillary columns that has caused so many chromatographers to use them wherever possible—and this example only begins to approach the potential of capillary columns for enhanced resolution and speed of analysis.

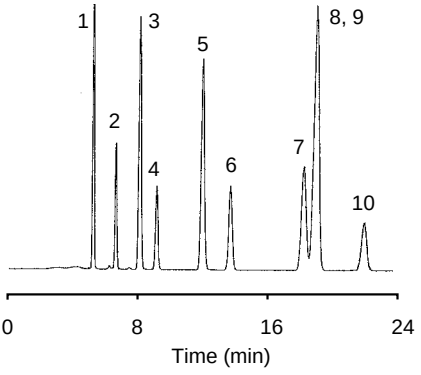
Of course, the capillary chromatogram in Figure 4.1b, obtained at 20 mL/min carrier-gas flowrate—a linear velocity of 172 cm/s—exhibits far less resolution than is possible when the flow or velocity is set closer to the optimum, as shown in



(*a*)



(*b*)



(*c*)

FIGURE 4.1 Packed/open tubular column comparison: (a) 2-m × 2-mm-i.d. packed column, 8% SE-30 on 80/100-mesh Chromosorb W-HP; helium carrier, 20 mL/min at 90°C; (b) 25-m × 0.53-mm i.d. × 5-μm film methylsilicone fused-silica column, 20 mL/min at 90°C; (c) same as (b), except 1.4 mL/min at 110°C. Peak identification: (1) *n*-pentane; (2) *n*-hexane; (3) benzene; (4) *n*-heptane; (5) toluene; (6) *n*-octane; (7) ethylbenzene; (8) *m*-xylene; (9) *p*-xylene; (10) *n*-nonane. [2]. (Reprinted with permission from *Introduction to Open Tubular Column Gas Chromatography*, p. 93, copyright © 1994 Advanstar Communications Inc. All rights reserved.)

Figure 4.1c. Now, at a velocity of 11.3 cm/s—close to that in the original packed-column run—the resolution increases dramatically, but the cost of obtaining the better resolution is a much longer elution time for all the peaks. In this third case the column temperature was increased by 20°C in order to reduce the last peak's elution time as well as improve the separation between ethylbenzene (peak 7) and the two xylenes (peaks 8 and 9).

Table 4.1 gives the fundamental chromatographic parameters and measurements from Figure 4.1. In particular, note that for the *n*-decane peak, the packed column actually has more plates per meter (1298) than does the capillary column (822) at optimum flow. It is the length of the capillary column that makes the difference in peak resolution, generating a total of 20,540 theoretical plates as opposed to only 2585 on the packed column.

The nonresolution of the two xylene isomers shows that brute-force resolving power is not always the most effective solution to a separation problem. A change of stationary-phase selectivity can be a much more effective approach to optimization; in this case a more polar stationary phase would be an appropriate choice. Not all chromatograms are so simple, however. A change of stationary phase may not be sufficient when there are many closely eluted peaks. Some pairs may experience improved resolution while others may actually be eluted closer together. This kind of complex separation is an ideal candidate for careful optimization of flow and temperature, as discussed later in this chapter.

TABLE 4.1 Comparative Data for Packed and Open Tubular Columns from Figure 4.1

	Packed	Wide-Bore Open Tubular	
Column	(A)	(B)	(C)
d_c (mm)	2.0	0.53	0.53
d_p (mm)	0.15–0	—	—
d_f (μm)	—	5	5
L (m)	2	25	25
T_c (°C)	90	90	110
Flow and pressure			
F_c (mL/min)	20	20	1.4
t_M (s)	20.8	14.5	220
$\overline{u}$ (cm/s)	9.6	172	11.3
p_i (psig)	30	18	1
Performance (last peak)			
t_R (s)	172	166	1029
k	7.3	10	3.7
w_h (s)	7.9	6.2	16.9
N (plates)	2585	3978	20540
N/L (plates/m)	1298	159	822
H (mm)	0.78	2.51	1.22

This simple illustration shows how the basic choice of capillary versus packed columns determines the nature of the resulting separation and how optimization of flow and temperature can produce further improvements. Now we will leave packed columns behind and consider in more detail the various avenues available for optimization of capillary-column separations.

4.3 THE INFLUENCE OF COLUMN VARIABLES

Capillary-column dimensions and the average carrier-gas linear velocity exert a strong influence on peak resolution and the speed of analysis. Chromatographers can control a separation's characteristics by choosing these parameters as required to meet specific performance goals. In order to simplify the discussion and to provide a clear separation of the variables' influences, we will hold the column temperature constant when considering the effects of changing the physical column parameters. The influence of column temperature is addressed later in this chapter.

4.3.1 Inner Diameter

The column inner diameter (d_c) strongly influences the minimum plate height (h_{min}) as well as the pressure drop (Δp) required to establish a specific average carrier-gas linear velocity ($\overline{u}$). These two influences produce profound changes in the chromatogram, as shown qualitatively in Figure 4.2, which compares (a) the 25-m $\times$ 0.53-mm-i.d. column from the previous example to (b) a 0.100-mm-i.d. column with the same stationary phase and length. A tremendous improvement in resolving power is quite evident, which stems from the much larger number of theoretical plates and the lower plate height, yet a 10-fold increase in pressure drop is required to bring the narrow-bore column linear velocity up to barely half that of the wide-bore column.

From this point on, and until we address directly the questions of column temperature and temperature programs, the column temperature will be assumed to be isothermal and the mathematical relationships given will hold for isothermal conditions only. The data in Figure 4.3 and Table 4.2 provide a quantitative look at the influence of the column inner diameter on the plate height as a function of the average carrier-gas linear velocity. According to theory, as the inner diameter decreases the minimum plate height also decreases, in the following manner:

$$h_{min} = d_c \sqrt{\frac{1 + 6k + 11k^2}{12(1 + k)^2}} \tag{4.1}$$

This relationship, although it neglects the influence of the stationary phase on band broadening, is clearly reflected in the experimental data, where smaller inner diameters produce lower minimum plate heights. The last column of Table 4.2 lists the utilization of theoretical efficiency (UTE%) for each column at its

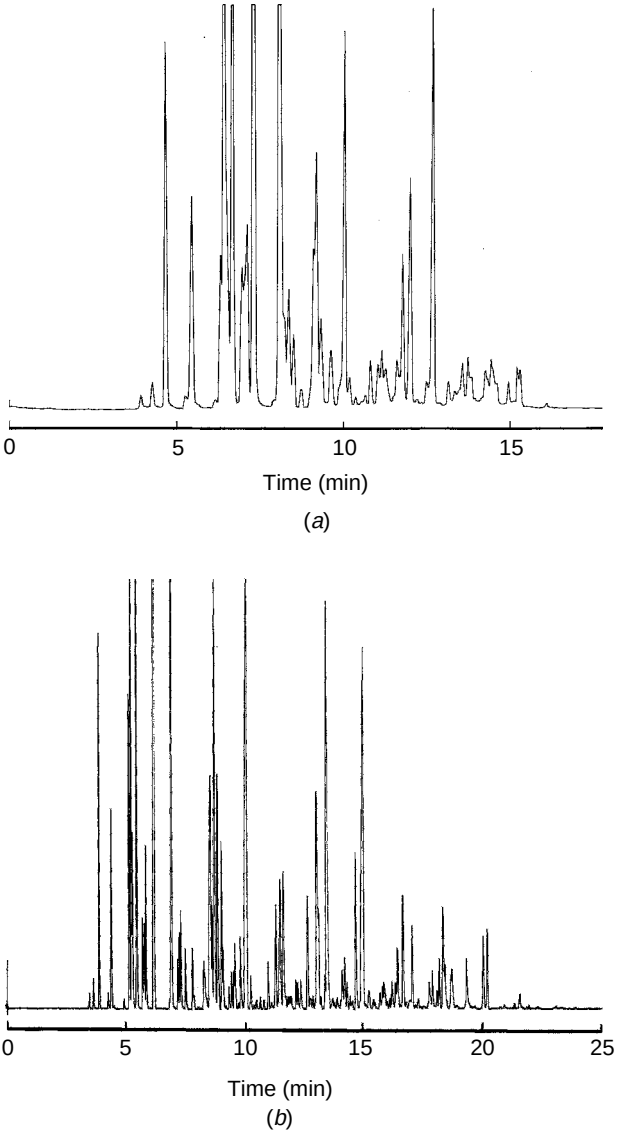


FIGURE 4.2 Effect of changing column diameter on peak resolution: (a) column: 25-m, 0.53-mm i.d., 5-μm methylsilicone film. Carrier: He at 5 psig, $\overline{u} = 30$ cm/s. Inlet: direct injection, 0.05 μL. (b) Column: 25-m, 0.100-mm i.d., 0.25-μm methylsilicone film. Carrier: He at 50 psig, $\overline{u} = 16.7$ cm/s. Inlet: Split ratio 100:1, 0.1 μL injected. Conditions (both chromatograms): oven, 30°C, hold 2 min, 3°C/min to 200°C. Detector: FID, 250°C. Sample: gasoline (3). (Reprinted with permission from *Introduction to Open Tubular Column Gas Chromatography*, page 101, copyright © 1994 Advanstar Communications Inc. All rights reserved.)

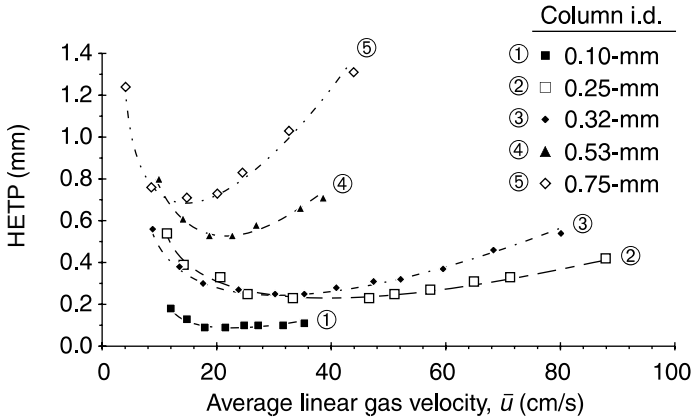


FIGURE 4.3 HETP vs. $\overline{u}$ plots for *n*-undecane on five open tubular columns, operated with helium carrier gas (4). (Reproduced from *The Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc. For analytical conditions, see Table 4.2.)

TABLE 4.2 Comparative Efficiency Data for Five Capillary Columns

d_c (mm)	L (m)	d_f (μm)	Phase Ratio, β	T_c (°C)	k for C_{11}	$\overline{u}$ (cm/s)	h_{min} (mm)	UTE%
0.10	25	0.09	278	110	1.63	20.0	0.069	77.1
0.25	25	0.25	250	110	1.81	38.5	0.178	80.8
0.32	25	0.26	308	110	1.47	30.2	0.217	86.8
0.53	25	5.5	24.1	130	9.69	15.0	0.472	66.5
0.75	30	1.03	170.5	130	1.28	20.5	0.491	94.4

Source: Reference 4.

optimum velocity (lowest plate height), which compares the calculated minimum plate heights from Equation 4.1 to the actual minimum plate heights measured from the chromatographic data in Figure 4.3. Here the influence of the stationary-phase film can be seen in the lower UTE% values for columns with thicker films.

A form of the Hagen–Pouseille equation gives the relationship between the inner diameter and the pressure drop [6]

$$\Delta p j' = \frac{32 L \eta \overline{u}}{d_c^2}$$
$$j' = \frac{3}{4} \cdot \frac{(P^2 - 1)(P + 1)}{P^3 - 1} \tag{4.2}$$

where j' is a compressibility correction factor to be applied to the average carrier-gas linear velocity, $P = p_i/p_o$ is the pressure ratio between the column inlet and

outlet, and η is the carrier-gas viscosity. The compressibility correction factor is greater than 0.85 for pressures below 505 kPa (74 psig), and it can be ignored for our purposes of illustration. Computer programs that perform such calculations, however, must include this influence if they are to be sufficiently accurate to make useful predictions.

This pressure–velocity relationship shows that the pressure required to produce a given average linear carrier-gas velocity through a column increases approximately as the inverse square of the diameter, as long as the column length, outlet pressure, and temperature are held constant. For the 0.53-mm-i.d. column mentioned previously, which requires 5 psig to produce an average velocity of 30 cm/s at 30°C, reducing the inner diameter to 0.25 mm will require roughly $(0.53/0.25)^2 = 4.5$ times as much pressure. Going down to 0.10 mm i.d., however, will require about $(0.53/0.1)^2 = 28$ times the pressure, which may well exceed the capacity of the inlet system. Hydrogen carrier gas, with about half the viscosity of helium, provides a lower-pressure alternative carrier-gas source that chromatographers can choose in such situations.

Gas chromatographic optimization usually implies a tradeoff of some kind when column dimensions and/or operation conditions are modified in order to achieve a specific goal. In the present example, the combined figures on the relationships of the minimum plate height and the pressure drop to the inner diameter indicate that reducing the column inner diameter while keeping the column length constant—in order to generate smaller plate heights and the concomitant higher peak resolution—will require pressure drops that increase as the square of the improvement in the plate height.

4.3.2 Length

Although no one has figured out how to change the inner diameter of a gas chromatographic column, it is easy enough to change the column length. Column length exerts a direct influence on retention time (speed of analysis), resolution, and pressure drop. In general, shortening a column while keeping the average linear velocity constant generates fewer theoretical plates and less peak resolution at shorter retention times, while requiring lower pressure drops. These trends are the inverse of reducing the column inner diameter, but their influence is not nearly as profound. However, in cases where peaks are overresolved—that is, when their widths are so narrow and separation factors are so large that a lot of extra time is spent waiting for them to be eluted—optimizing the column length can increase the speed of analysis several times over. As long as a minimum critical pair resolution is maintained, this kind of length optimization can yield faster analyses and reduce column costs as well.

Figure 4.4 illustrates the effects of reducing length by successively halving a column until resolution of a critical peak pair drops below a minimum of 2.0. A minimum resolution level of 1.5 may be too low because it does not allow any overhead for eventual column degradation or other loss of performance. The

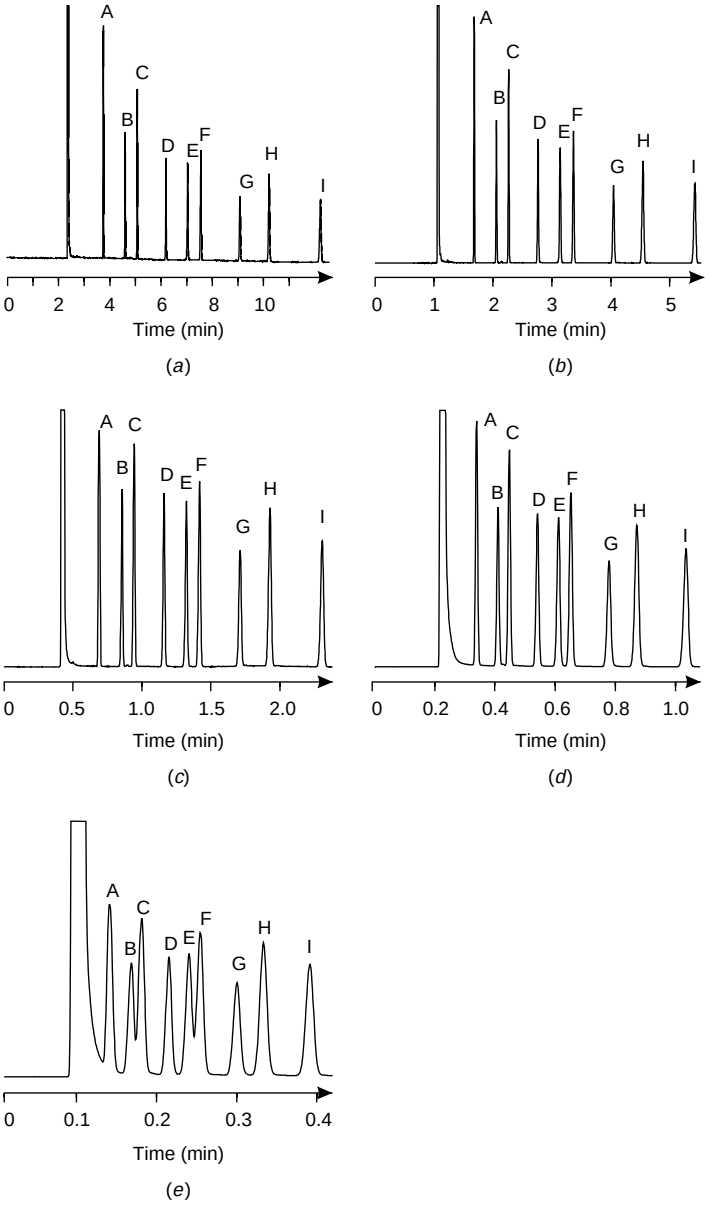


FIGURE 4.4 The effect of column length on peak resolution. Nominal column lengths: (a) 50-m, (b) 25-m, (c) 12-m, (d) 5-m, and (e) 2.5-m, 0.25-mm i.d., 0.25-μm methylsilicone. Conditions: helium carrier gas, 100°C oven, split injection, FID detection. Peak identification: (a) *n*-nonane, (b) 2-octanone, (c) *n*-decane, (d) 1-octanol, (e) 2,6-dimethylphenol, (f) *n*-undecane, (g) 2,4-dimethylanaline, (h) naphthalene, (i) *n*-dodecane. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (5).]

initial chromatogram (a), which was obtained on a 50-m column, overresolves all the peaks, and requires more than 15 min for the last peak to be eluted. As the column length is repeatedly cut in half, the retention time is reduced by the same amount, and the peak resolution also decreases. In this example there are two critical peak pairs: B–C and E–F. For the second pair, the resolution drops to 2.42 at a column length of 5 m. At this length, while all peaks are still fully resolved, the last peak's retention time has dropped by a factor of 12 from more than 15 minutes to just over one minute. Going down to 2.5 m column length further reduces the retention times, but now there is no longer sufficient resolution to completely separate all the peaks.

The effect of length on retention time can be expressed in the following relationship:

$$t_R = \frac{L}{\overline{u}}(k+1) \tag{4.3}$$

Decreasing the column length while keeping the average carrier gas linear velocity constant will decrease retention times in the same proportions. As for the peak resolution, the resolution equation can be written in terms of the column length, and the plate height and retention factor of the second of a pair of peaks in this way:

$$R_s = \frac{1}{4}\sqrt{\frac{L}{h_2}} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k_2}{k_2 + 1} \tag{4.4}$$

This equation assumes that the peak widths of adjacent closely eluted peaks are essentially the same. As long as the plate height is not affected significantly by reducing the column length, then the loss of resolution incurred by shorter columns is only the square root of the length reduction. Thus, for each time that a column is halved, the resolution should be reduced by $\sqrt{2}$. The data in Table 4.3 approximate this relationship, although the resolution losses are somewhat greater than Equation 4.4 predicts. This greater resolution loss, which occurs only on the shortest columns, can be attributed to the peaks becoming narrower than the detector time constant or inlet bandwidth can accommodate.

TABLE 4.3 Data for Various Column Lengths from Figure 4.4

L (m)	t_M (min)	$\overline{u}$ (cm/s)	t_R, n-C_{12} (min)	R_s 2-octanone/n-C_{10}
50.4	2.83	29.7	15.33	11.24
24.9	1.38	30.1	6.88	7.65
12.5	0.58	35.8	2.89	3.71
4.9	0.26	32.0	1.09	2.42
2.5	0.12	35.2	0.50	0.86

Source: Reference 5.

4.4 THE INFLUENCE OF OPERATIONAL VARIABLES

The selection of a column's physical dimensions determines its fundamental separation characteristics as well as the instrumental and operational requirements for obtaining a desired level of performance. Column physical parameters are not easily changed. Generally it is not convenient or expedient to adjust a column's length once selected and installed, and replacing one column with another that has a different internal diameter is not often practical or economic. Thus, once a column has been selected, further separation optimization can take place within the realm of the operational variables of carrier-gas flow or velocity, and the column temperature or temperature program.

4.4.1 Carrier-Gas Linear Velocity

The carrier-gas linear velocity influences both peak resolution and retention time in a manner that is the inverse of the influence of the column length; that is, higher velocities reduce retention times, and if increased significantly above the optimum level, they will also reduce resolution. Choosing higher linear velocities is often the best course for obtaining faster analysis speeds if it is not practical or desirable to select a smaller column inner diameter or to reduce the length. The useful range of linear velocities is more restricted by the choice of length and inner diameter in relation to the inlet pressures that the instrumentation can provide. In addition, as peaks become narrower at higher velocities, detector response times may be insufficient to transduce their shapes accurately.

The relationship between retention time and the linear velocity has already been given in Equation 4.3. Simply put, increasing the linear velocity will reduce all peak retention times proportionately. It is easy to increase a chromatogram's speed above the optimum velocity by raising the inlet pressure. Figure 4.5 shows a series of chromatograms obtained at incrementally higher average carrier-gas linear velocities; as the velocity increases, the peaks' retention times decrease accordingly, by a factor of approximately 10 from the slowest to the fastest chromatogram.

In this example, which uses a wide-bore column, the inlet pressures for these velocities range from about 1 psig to just over 10 psig helium, so there is no difficulty in achieving the desired range of velocities. With narrower-bore and longer columns, however, the upper pressure limit of many carrier-gas supplies may curtail the available range of linear carrier-gas velocities. For example, a 50-m $\times$ 200-μm-i.d. column will require around 40 psig of helium carrier to achieve a 30 cm/s velocity at 50°C, but in excess of 160 psig will be needed to push the velocity to 110 cm/s. Thus, going to faster chromatography with narrower and longer columns may require specialized pneumatic systems that are capable of delivering much higher carrier gas pressures. Another approach to achieving higher velocities involves substituting hydrogen carrier for helium carrier gas, which will produce approximately twice the average linear velocity at the same pressure because of the reduced viscosity of the hydrogen carrier gas.

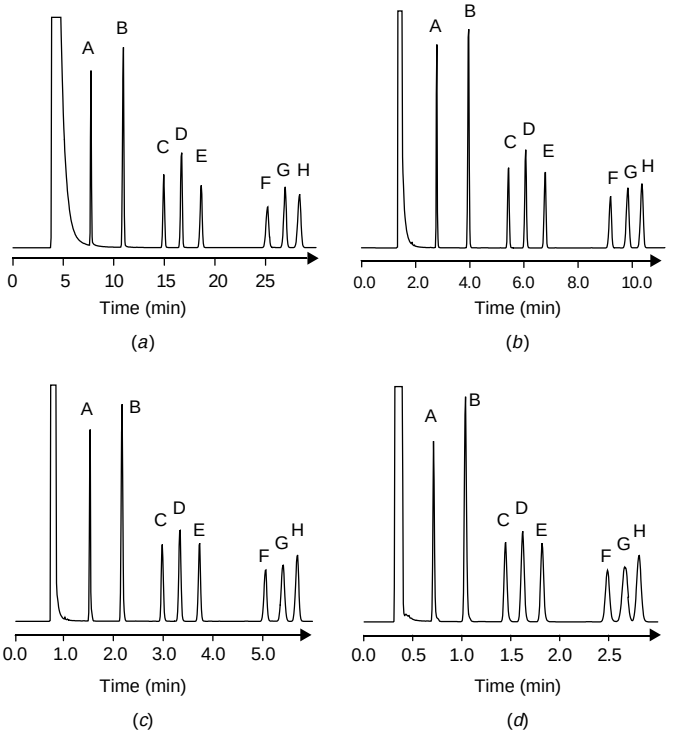


FIGURE 4.5 A series of chromatograms at increasingly higher average carrier-gas linear velocities. Velocities: (a) 11 cm/s; (b) 30 cm/s; (c) 53 cm/s; (d) 105 cm/s. Sample: A = *n*-nonane, B = *n*-decane, C = 1-octanol, D = *n*-undecane, E = 2, 6-dimethylphenol, F = 2, 4-dimethylaniline, G = *n*-dodecane, H = naphthalene; 2 μg/mL each in isopropanol (4 μg/mL *n*-decane). Column: 25-m × 0.53-mm-i.d. 3-μm film thickness 5% phenylmethylsilicone, He carrier at 125°C, packed inlet with on-column adapter at 200°C; flame ionization detector at 200°C, range 1. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (7).]

However, the use of hydrogen carrier must be considered carefully in terms of individual lab safety requirements. In some labs, hydrogen is preferred because it is less expensive than helium.

In Chapter 3 we have seen that the column efficiency depends on the average carrier-gas linear velocity according to well-defined theoretical relationships of the van Deemter–Golay equation. How this dependency affects these example separations is shown in Figure 4.6 as a plot of the measured and the theoretical plate heights versus the average carrier-gas linear velocity for one of the peaks shown in the examples in Figure 4.5. From a minimum of about 0.6 mm at the optimum velocity of 18 cm/s the plate height increases to 3.2 mm at the maximum experimental velocity of 110 cm/s. The slight positive displacement of the experimental data above the theoretical data at the higher linear velocities

may be attributed to slow detector response to the smaller peak widths at these elevated speeds. In Figure 4.6, the theoretical plate height calculations include the band-broadening effects of the 3-μm stationary-phase film.

These increasing theoretical plate heights lead in turn to a loss of peak resolution at the higher velocities. Figure 4.7 illustrates the measured peak resolution

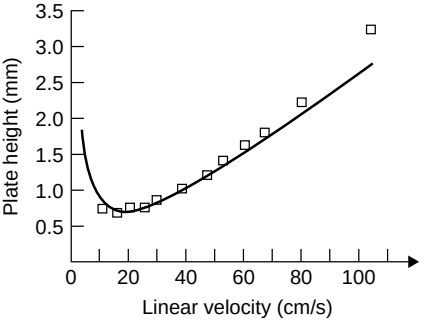


FIGURE 4.6 Plot of plate height (H) versus the average carrier gas linear velocity ($\overline{u}$). The line represents the theoretical plate height calculated from the column dimensions and stationary-phase film thickness; the squares are the measured values for the *n*-dodecane peak as determined from a series of chromatograms, several of which appear in Figure 4.5. For conditions, see Figure 4.5. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (7).]

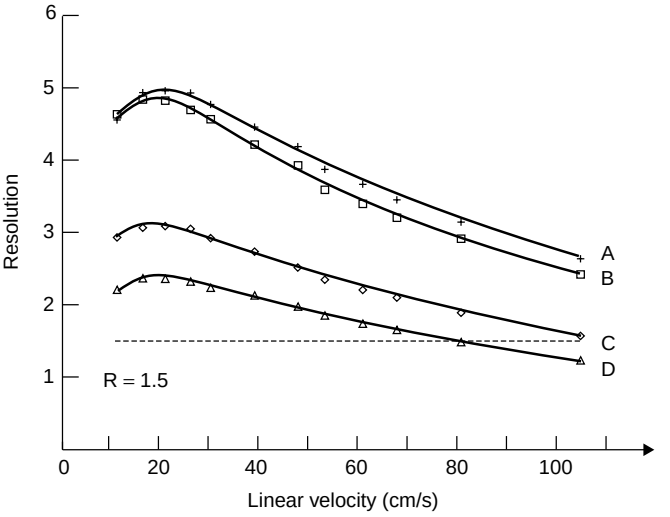


FIGURE 4.7 Plot of resolution versus average carrier gas linear velocity. The lines represent resolution calculated from theory, and the points represent the measured values for the following pairs of peaks: A = *n*-undecane, 2,4-DMA; B = 1-octanol, *n*-undecane; C = 2, 4-DMA, *n*-dodecane; D = *n*-dodecane, naphthalene. For conditions, see Figure 4.5. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (7).]

for four pairs of peaks in this series of chromatograms. The resolution drops off at linear velocities under about 20 cm/s, due primarily to gas–gas diffusion–based broadening in the mobile phase. The velocity that generates optimum resolution varies slightly for each peak pair because each peak reaches its minimum plate height at different average linear velocities: Peak pairs A and B are the best examples of this effect. As the linear velocity increases above 30 cm/s the resolution starts to fall off. The resolution of peak pair D (dodecane/naphthalene) drops below 1.5 (baseline-level resolution) at velocities above 80 cm/s. As the least-resolved pair of peaks in the analysis, pair D is deemed the *critical pair* because its behavior determines the optimum tradeoff between speed and resolution. All the other pairs of peaks exhibit higher resolution than does the critical pair.

Thus, for the current example a linear velocity of 80 cm/s produces the fastest chromatogram that still resolves all peaks with at least baseline resolution. Many chromatographers choose operating points that allow some leeway for column degradation over time as well as accommodate unknown peaks or unexpected baseline disruptions. Operating this separation at 40 cm/s, for a minimum resolution of 2.0 for critical peak pair D, will allow some leeway for such events but will sacrifice fully half of the available speed of analysis.

4.4.2 The Influence of Column Temperature

Up to this point we have discussed the optimization of gas chromatographic separations by manipulation of the column variables that do not affect peaks' relative retentions. Changing the column dimensions, the stationary-phase film thickness or the carrier-gas velocity will affect retention times, but the peaks' thermodynamic partition coefficients (K) remain constant a long as the column temperature and the stationary-phase chemistry remain unchanged. As a result, the peaks' relative retentions—the ratios of their adjusted retention times (t'_R)—also will not be affected by such manipulations, and so the peaks' elution order and relative separations remain unchanged. This makes prediction of the effects of modifying these variables fairly simple to compute using relationships such as those presented thus far in this chapter.

The range of separation effects that chromatographers can produce is greatly expanded when the column temperature comes into play. However, the relationship of retention time to column temperature is nonlinear, and individual peaks are not equally affected by temperature shifts. In fact, as we shall see, peaks often will merge or even reverse their elution order as isothermal temperatures or temperature programs are modified. These more complex thermal relationships combine with the influences of the column physical dimensions and the carrier-gas flow to make a fully delineated model of a particular separation beyond the capability of many chromatographers to construct using tools such as a scientific calculator or a spreadsheet. Computerized models of separation behavior can provide the necessary functionality for fully enabled optimization.

4.4.2.1 Isothermal Operation

In general, retention times become shorter as the column temperature increases, primarily as a result of increasing solute vapor pressures. The column temperature also influences solute-specific interactions, such as polarizability, hydrogen bonding, and steric hindrance, which gives rise to differential effects and causes solute relative retentions to change with temperature.

Figure 4.8 illustrates both effects for a capillary column test mixture. At 90°C, dodecane is eluted last at around 11 min. As the temperature increases in 10° increments, all of the peaks' retention times decrease, and the entire separation takes only 3.6 min at 120°C. The solutes' retention times decrease by about half for every 15–20°C increase in column temperature. However, the last two peaks merge at 100–110°C, and naphthalene becomes the last peak at 120°C. Thus, careful attention must be paid to unambiguous peak identification during a separation optimization that includes the column temperature.

We can illustrate the various peaks' retention behavior as a function of temperature by making a plot such as shown in Figure 4.9, which presents the log of the retention factor as a function of the reciprocal of the (absolute) column temperature from 75 to 130°C. This type of relationship can be characterized by two constants as shown in the following equation:

$$\log(k) = \frac{A}{T_c} + B \tag{4.5}$$

Each peak has its own A and B constants. The straight lines in Figure 4.9 show the result of fitting the data to such an equation, and they demonstrate that this relationship does very well in predicting the influence of the column temperature on isothermal separations. In fact, only two retention measurements at different temperatures would be required to characterize the temperature relationship. Generally the most accurate A and B values for each peak are obtained by choosing a minimum and maximum temperature plus one additional temperature in the middle and then interpolating to a target temperature value.

A log (k) versus $1/T$ plot very nicely demonstrates that we can predict accurate peak retention factors as a function of isothermal column temperature. However, the separation of those peaks is of more interest for optimization. We can observe from the plot that the naphthalene and dodecane peaks will merge between 100 and 110°C, but it is difficult to imagine at what temperature or temperatures all of the peaks exhibit maximum separation. In order to better visualize these relationships, Laub and Purnell (9,10) described a *window diagram* plot of the peak's separation factors (α), which can be constructed from retention factor data such as presented in Figure 4.9 according to

$$\alpha = \frac{k_{i+1}}{k_i} \tag{4.6}$$

for the ith and subsequent peaks. The separation factor equals one when two peaks are coeluted. Such plots make it easy to see which peak pair is the least

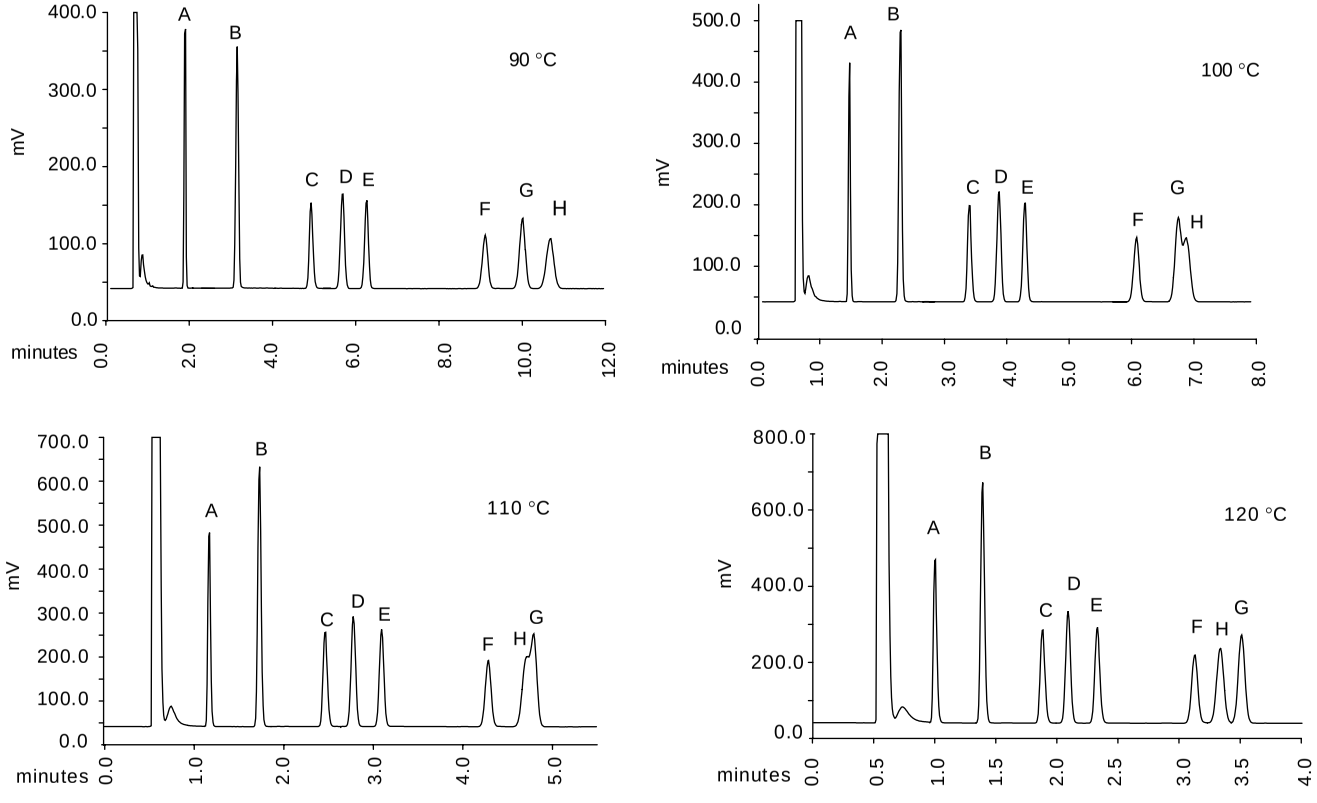


FIGURE 4.8 The influence of isothermal column temperature on separation. Column temperature: as shown. Sample: 0.1 μL direct injections of a 2 μg/mL 1-propanol solution of A = *n*-nonane; B = *n*-decane; C = 1-octanol; D = *n*-undecane; E = 2, 6-DMP; F = 2, 4-DMA; G = naphthalene; H = *n*-dodecane. Column: 30-m × 0.53-mm i.d. × 3-μm 5%-diphenyl-95%-dimethylpolysiloxane stationary phase. Helium carrier at constant 12 mL/min flow. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (8).]

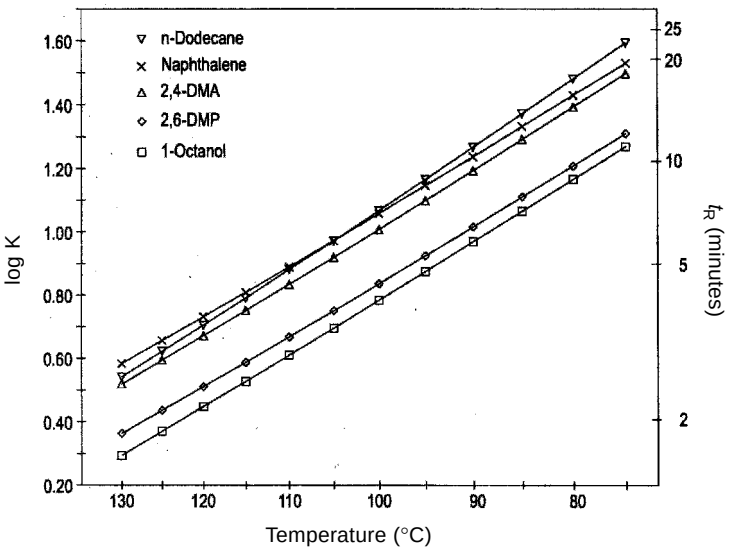


FIGURE 4.9 Plot of log(k) and t_R versus $1/T_c$ for five peaks at various temperatures. The individual points represent experimentally measured retention values; the lines represent best-fit linear regression of the experimental points for each component. For conditions, see Figure 4.8. The temperature scale indicates degrees Celsius for clarity of presentation; the data are plotted against reciprocal degrees Kelvin. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (8).]

separated at a specific column temperature as well as to find the point(s) of maximum separation.

Figure 4.10 shows a window diagram plot of the separation factor and the isothermal column temperature for the present series of chromatograms, for the three closest-eluted peak pairs. Two discontinuities exist at close to 105°C where the naphthalene and dodecane peaks cross each other. Below this temperature, the elution order is dimethylanaline (DMA), naphthalene (NAP), and dodecane (C12). Above it, the order becomes dimethylanaline, dodecane, and naphthalene. The separation factor is not defined for nonadjacent peaks, so the plot is truncated at the coelution temperature where the elution order changes. The white areas in the plot represent the "windows" in which the optimum column temperatures exist. There are two separation factor optima for this set of solutes. The optimum with the largest separation factor lies at 85°C, and the next highest at 122°C. Taken alone without consideration for other influences, 85°C appears to be the best choice. If higher speeds are desired, then operating the column at 122°C may work, but information that would help to make such an assessment is missing from the plot. We do not know what minimum separation factor is required in order to deliver a specific minimum desired resolution. Also, note that the separation factor window diagram plot is independent of the carrier-gas flow as well as of the column physical parameters. It depends only on the physicochemical relationships of the solutes to the stationary phase.

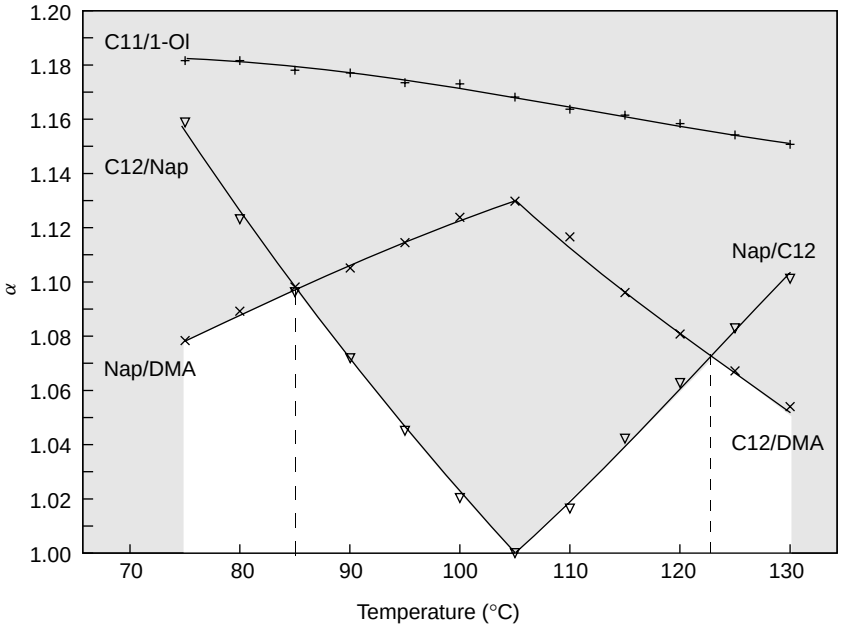


FIGURE 4.10 Window diagram plot of separation factor (α) versus column temperature. Derived from the retention factor data in Figure 4.9 [Reprinted from *LC/GC Magazine* with permission of Advanstar Publication (8).]

Of course, the objective is to resolve the solutes, not merely separate them, and Figure 4.10 tells us nothing about the degree of peak resolution that can be expected. The resolution *will* depend on the carrier-gas flow and the column physical parameters because the peak widths and thus the theoretical plate height influence the observed resolution. In order to characterize peak resolution across a range of column temperatures, we must either perform a series of experiments and measure resolution directly, construct an empirical mathematical model by fitting curves to a smaller dataset, or find a model that encompasses the additional variables and requires a minimum amount of experimental data.

Figure 4.11 illustrates the first, "brute force" approach, based on the resolution data that are already at hand for the present example. This resolution-based window diagram strongly resembles the separation factor window diagram of Figure 4.10, but there are several significant differences. First, and perhaps most importantly, we can now gauge how well the peaks are resolved across the temperature range. The windows have been truncated in order to express a minimum required resolution of $R = 1.5$, which has caused the right-hand window to disappear entirely. A 90°C operating temperature produces the desired peak resolution. The maximum resolution obtained in the higher-temperature region is nearly 1.5 at around 120°C, and if some sacrifice of baseline resolution for the naphthalene/dodecane peak pair is acceptable in exchange for a faster speed

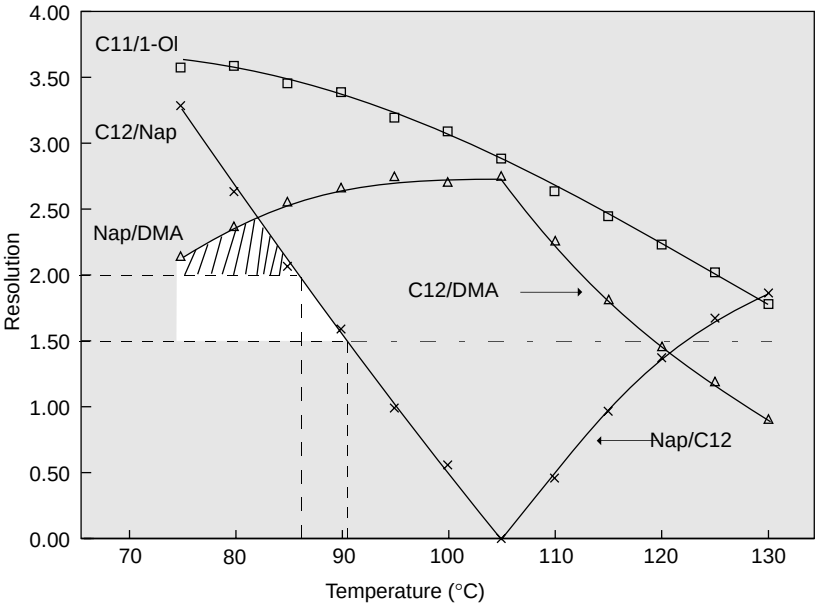


FIGURE 4.11 Window diagram plot of resolution (R) versus column temperature. Derived from the retention factor data in Figure 4.9. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publication (8).]

of analysis—well under 5 min compared to ~12 mins at the lower temperature—then we might choose the higher temperature. On the other hand, if we desire a higher minimum resolution of 2.0, as shown in the hatched window area, we must restrict the operating temperature range to between 75 and 85°C, with the higher temperature giving a somewhat faster speed of analysis. In this case there is no option in the higher temperature ranges. Instead, in order to attain higher speeds and still resolve the peaks, it would be necessary to choose a different column with a smaller inner diameter that can provide the necessary higher theoretical plate numbers in a similar analysis time.

Another significant difference between the separation factor window diagram and the resolution window diagram is the location of the maxima, which are both shifted to about 2–3°C lower temperatures when considering resolution. These differences, while not large enough to be significant overall, show how the resolution depends on the peaks' dispersive as well as retentive behavior. The third difference in the plots is the significantly increased curvature in the resolution window diagram plots, which also reflects the interplay of all the gas chromatographic variables with the resolution, as opposed to the simpler effects that give rise to the observed separation factors.

Although a resolution window diagram such as Figure 4.11 presents a much more useful view of the overall separation, its construction is more complex than a simple separation factor window diagram; it requires measurement of both

retention times and peak widths. The brute-force approach taken here required 12 separate chromatograms, one at each temperature, and then all of the retention and peak width data had to be processed in the data-handling system and exported into a spreadsheet. Once constructed, such a spreadsheet can be reused for other situations: a dedicated computer program would serve just as well. A less intensive approach to obtaining this information involves acquiring a smaller number of chromatograms—say, one for every 10° instead of every 5°—and then interpolating to estimate the intermediate values. As the number of data points decreases, of course, the accuracy of the interpolation suffers, especially when there is significant curvature involved. The third approach—developing mathematical models of the elution process—is the topic of this chapter's section on computerized optimization.

4.4.2.2 Temperature-Programmed Operation

Isothermal operation limits the range of solutes that can be eluted within a reasonable time limit, due principally to the large decreases in solute volatility that occur with increasing solute molecular weight. In order to broaden the scope of separated compounds, chromatographers often resort to changing the column temperature during the elution process, or *temperature programming*. By far the most common temperature programming method increases the temperature of the entire column until all peaks have been eluted. Other temperature-programming techniques, such as chromathermography (11,12), which involves the passage of a moving temperature field along the column, have not received as much attention.

What happens to the elution process as the temperature is changed? We already know that retention times decrease with increasing temperature, approximately halving with each 15–20° increase. We also know that this effect varies slightly from one peak to another, giving rise to observed changes in relative retention and peak resolution. Elution under programmed-temperature conditions can be modeled by imagining that the column temperature is changing in discrete steps of, say, 10° every minute. One minute after injection the temperature "instantly" jumps up 10°. After 2 min, it jumps another 10°. So during the first minute the peaks move down the column according to their retention behavior at the initial temperature. During the second minute they move according to the 10° higher temperature, and during the third minute, 20° higher. Now, if we know the peaks' isothermal retention factors at two temperatures, we can use equations from isothermal elution to predict how far each peak moves during the first, second, and third minutes and so on, until all the peaks have eluted from the column.

For example, suppose that we are using a 10-m column with a linear velocity of 33 cm/s at 50°C, and that peak 1 elutes isothermally in 10 min, with a retention factor (k) of

$$k = \frac{t_R - t_M}{t_M} = 19 \tag{4.7}$$

Assuming for simplicity that the linear velocity is constant along the entire length of the column, in one minute the peak will have moved one-tenth of the column length (1 m). Now, suppose that the peak would be eluted in 7 mins at 60°C.

In the second minute the peak will therefore move another one-seventh of the column (1.4 m), and its retention factor will equal 13. After the first 2 mins the peak will have moved a total distance of

$$z_t = 1.0 + 1.4 = 2.4 \text{ m} \tag{4.8}$$

In this equation z_t is the total distance that the peak has moved along the column at time t. We can use these two sets of retention data to predict the peak's behavior at higher temperatures by recalling the linear relationship of $\log(k)$ to the reciprocal of the absolute temperature in Equation 4.5 as discussed earlier in this chapter.

Using temperature and retention factor data for 50 and 60°C, we can compute the coefficients A and B in Equation 4.5 for this example, and then apply them to higher temperatures in order to predict isothermal retention times and retention factors. These data can then be used to predict how far the peak will move during each discrete temperature step in our example. A spreadsheet program was used to calculate this data as shown in Table 4.4. The values for k and t_R are for isothermal elution at the indicated temperature step. The values for z_t are the total distances the peak has moved at the end of the indicated step. The last entry for z_t shows that the peak has been eluted from the column ($z_t = 10.0$ m) in just less than 5 min during the 90°C temperature step.

In reality it is neither practical nor desirable to step the column oven temperature in 10° increments every minute, nor does the stepwise model predict elution times with sufficient accuracy for our purposes. If we now imagine instead that the oven temperature increases in 1° steps every 6 s or even better in 0.1° increments every 600 ms, we can approach a true linear temperature program rate of 10°C/minute as is encountered in modern gas chromatographic systems. Our isothermal retention data at 50 and 60°C are still valid, and we could calculate the peak positions for each 0.1°C step in a tabular format. The problem is that even this small a step is still too large for accurate prediction of programmed-temperature retention times. Instead, we must to turn to calculus and consider an arbitrarily small step size (dt). A simplified relationship of a single-step linear temperature program to elution time can be expressed as follows [13]:

$$1 = \int_0^{t_R} \frac{1}{t_{M,t}(k_t + 1)} dt \tag{4.9}$$

TABLE 4.4 Predicted Retention Behavior for Stepwise Temperature Programming

Time (min)	Temperature (°C)	k (isothermal)	t_R (isothermal)	z_t (meters)
0.0	50.0	19.0	10.0	1.0
1.0	60.0	13.0	7.0	2.4
2.0	70.0	9.1	5.0	4.4
3.0	80.0	6.5	3.7	7.1
4.0	90.0	4.7	2.9	10.6

Here k_t is the retention factor from equation 4.5 expressed as a function of the temperature program, and $t_{M,t}$ expresses the effect of changing temperature on the unretained peak time. This type of calculation is conveniently carried out on a personal computer using either a commercially available elution prediction program or a spreadsheet, and we will discuss it in more detail in the next section on computerized optimization.

The ability of programmed-temperature elution to deliver faster speeds of analysis can be understood from the preceding stepwise example. Peak 1, which has an isothermal retention time of 10.0 min at 50°C, is eluted in less than 5 min with temperature programming from 50 to 90°C at 10°C/min. The timesaving on longer columns is much greater. For example, the same peak would be eluted in 50 min at a linear velocity of 33 cm/s on a 50-m column. At a program rate of 10°C/minute, the peak would come off the column at about 145°C, in under 11 min, or in about 22% of the isothermal retention time. Choosing a less volatile component, say, one with $k = 100$ at 50°C, we find that it has an isothermal retention time of about 252 min. With temperature programming at 10°C/min, this peak would be eluted at around 210°C in less than 17 min, or in about 6.7% of the isothermal time. These values are approximate, since the stepwise spreadsheet model in Table 4.4 was used in the calculations, but they indicate the degree of timesaving that temperature programming offers.

The oven cooldown time that is required to return the gas chromatographic oven to its initial temperature after an analysis must also be considered. Most commercial gas chromatographs will cool from 250 to 50°C in less than 6 min. Adding a 2-min temperature equilibration time before the next analysis can be started, we can expect a total time penalty of around 8 min for temperature programming the oven. When compared to an improvement of 39–200 min over the isothermal situation for this hypothetical 50-m column, the cooldown time is insignificant. For shorter columns and under higher-speed temperature programming conditions, however, the cooldown time may become a limiting factor. Developments in column temperature programming hardware, such as resistively or microwave-heated columns, engender much shorter cooldown times and make high-speed temperature-programmed analysis much more practical.

We have examined the effects of carrier-gas linear velocity and temperature on isothermal gas chromatographic separations in the previous two sections of this chapter. In both cases it was important to consider not only peaks' relative retentions or separation factors but also their resolution. When changing column flowrates or linear velocities, the peaks' relative retentions remained constant and the peak widths changed. By establishing a minimum acceptable resolution of 1.5 and a desired resolution of 2.0, an optimum flow or velocity could be selected on the basis of achieving the resolution goal in a minimum time. When changing the isothermal column temperature, peaks' relative retentions did not remain constant. Peaks shifted with respect to each other, and one pair in our example was coeluted at a temperature midway through the experimental range. The optimum temperature was selected on the basis of minimum time to achieve the desired minimum resolution goal.

Optimization of programmed-temperature elution is a similar problem. As is the case in isothermal temperature optimization, the peaks shift relative to each other as the program rate is changed. Higher programming rates cause elution temperatures to increase, but peak retention times decrease because it takes less time to reach the elution temperature. Thus, the criteria of achieving a resolution goal in the minimum time can also be applied to temperature programming.

In order to investigate programmed-temperature optimization, a series of runs were made using a pesticide calibration mixture with the program rates ranging from 1.5 to 30°C per minute. Four example chromatograms from this series are shown in Figure 4.12. As the program rate increases from 1.5°C/min (Figure 4.12a), the peaks move relative to each other. At 4°C/min (Figure 4.12b), the peaks labeled J and K have reversed elution order, and the elution time of the last peak has dropped from about 60 min to about 28 min. As the rate increases to 8°C/min (Figure 4.12c), peaks P and Q are now separated. Peaks D and E move closer to each other with increasing programming rates, until they are coeluted at 20°C/min (Figure 4.12d). The runtime decreases to just over 12 min at the highest rate.

The previous examples of isothermal temperature and flowrate optimization used simple plots of resolution versus temperature or linear velocity to visually determine the optimum parameters for the two or three least-resolved, critical peak pairs. The mixture used in the present study is too complex for facile visualization of the optima in this manner. An alternative plot can be used in such cases, shown in Figure 4.13, which presents a plot of resolution against program rate for each of the adjacent peak pairs in the example. It includes data for all the measured program rates (as shown on the x axis). In Figure 4.13, resolution is represented by the degree of shading of the bands. A dark band indicates coelution of the two associated peaks. A medium gray band indicates overlapping peaks with resolution between 1.01 and 1.5. A light gray area indicates completely resolved peaks (resolution greater than 1.5). Figure 4.13 is a two-dimensional projection of a three-dimensional plot in which the third, or z, dimension is represented by depth of shading or color.

Some of the peaks shown in Figure 4.13 shift and reverse their relative positions. The left-hand side of the figure lists the peaks in elution order at 1.5°C/min, while the right-hand side lists them in their elution order at 30°C/min. Peaks J and K (dieldrin and p, p'-DDE), for example, are partially resolved at 1.5°C/min, are coeluted at 3°C/min, and emerge in reverse order at 4°C/min and higher. The optimum program rate in this example lies at around 8°C/min, with a total elution time of about 17 min. Higher or lower rates do not meet the criteria for resolution and speed. For example, 6°C/min provides essentially the same resolution as the optimum, but the runtime is longer. At 12°C/min peaks D and E are coeluted, although better resolution is obtained for the rest of the peaks. One or more pairs of peaks are coeluted at all the rates below 6 and above 12°C/min. Here again, as we found with the previous isothermal elution example, it would be necessary to choose either a longer column or one with a smaller inner diameter in order to attain higher speeds and still resolve all the peaks of interest.

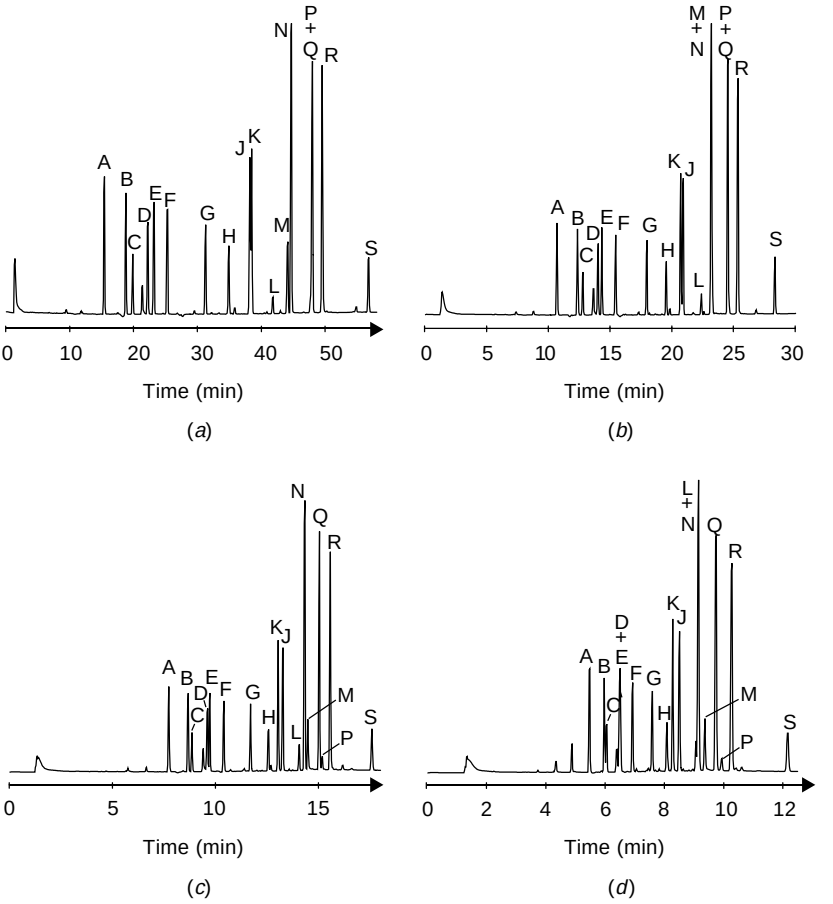


FIGURE 4.12 Effect of temperature program rate on the separation of a pesticide calibration mixture for United States Environmental Protection Agency (USEPA) method 608 at (a) 1.5, (b) 4.0, (c) 8.0, and (d) 16.0°C/minute. Conditions: 25-m × 530-μm-i.d. 65% methyl–35% phenylsilicone fused-silica column; constant-flow controlled helium carrier gas at 3.5 mL/min., temperature-programmed from 50 to 275°C; direct injection of 0.5 μL of 60–200 pg/mL each component in methanol at 300°C; electron-capture detection at 350°C, range 1 × 64. Sample (identifications according to sample manufacturer): A = α-BHC, B = γ-BHC, C = β-BHC, D = heptachlor, E = δ-BHC, F = aldrin, G = heptachlor epoxide, H = endosulfan-I, J = dieldrin, K = *p*, *p*′-DDE, L = endrin, M = endosulfan-II, N = *p*, *p*′-DDD, P = endrin aldehyde, Q = *p*, *p*′-DDT, R = endosulfan sulfate, S = decomposition product. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (14).]

4.5 THE ROLE OF COMPUTERS IN OPTIMIZATION

The optimization of gas chromatographic temperature programs is similar to optimization of isothermal temperature or carrier-gas linear velocity; criteria that

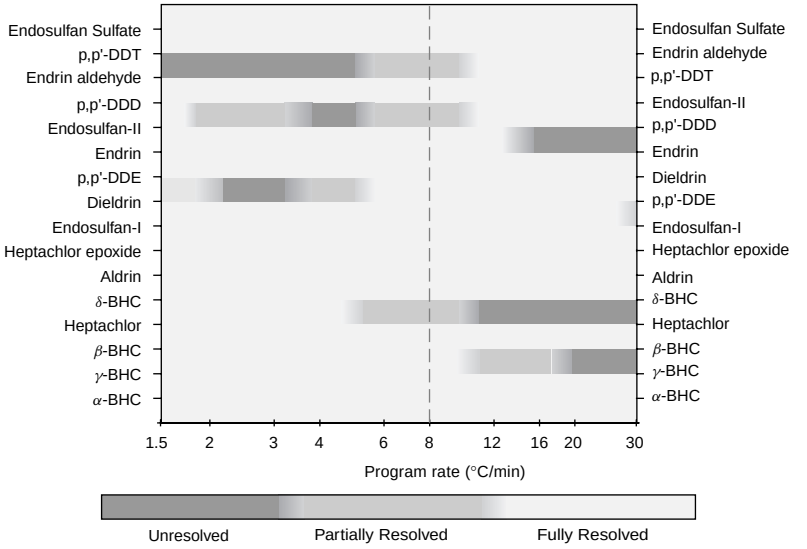


FIGURE 4.13 Peak resolution as a function of program rate for peak pairs in the pesticide test sample. For conditions, see Figure 4.11. [Reprinted from *LC/GC Magazine* with permission of Advanstar Publications (14).]

identify one or several optima for minimum resolution and analysis time can be established by the analyst and applied to the task of selecting an optimum set of operating conditions. However, information-rich modeling of peak resolution as a function of column dimensions, carrier gas, and thermal conditions is a complex task not easily engaged in without the assistance of specific computational tools. A number of computer programs are available that can help alleviate this complexity and facilitate visualization of the effects of changing the variables. In addition, such programs perform various types of optimization calculations directed toward achieving a set of defined goals.

Optimization by computer program is based on two fundamental components: a system model and an optimization engine. The system model provides information about how a system—chromatographic peak elution in this case—behaves as the independent variables change. The optimization engine uses the system model to compute sets of variables that most closely achieve a desired goal. For gas chromatographic optimization the independent variables are the familiar list of column dimensions, stationary-phase chemistry, carrier-gas pressure, and column temperature conditions. Other influences that are external to the column, such as injection bandwidth or detector response times, may come into play in simulations that also render peak shapes. Optimization results rely on the accurate input of one or more sets of real chromatographic retention times and conditions. If the input data were inaccurate, predicted results might not mimic real-world chromatograms closely enough to be useful. Secondary to the input data, the system model must encompass sufficient degrees of freedom and response functionality

to ensure accurate results prediction. Semiempirical structure–activity relationships sometimes are used as an alternative basis for a chromatographic system model; this topic lies outside the scope of the present discussion.

Computer-based optimization engines range from a minimum resolution threshold calculation through window diagrams to more complex simplex optimization schemes. They propose sets of conditions that satisfy the chromatographer's optimization criteria. The chromatographer operating the software often plays the role of the optimization engine by examining the results of gas chromatographic simulations across a range of variables and then picking out the most suitable conditions. Ultimately, the analyst must decide which conditions seem the best and then must evaluate that decision by making injections and assessing the results.

4.5.1 System Models for Optimization

Mathematical models for isothermal and for programmed-temperature gas chromatographic optimization are similar; many programmed temperature models build on thermodynamic information obtained from isothermal chromatographic data, although some use programmed-temperature input data to characterize the separation thermodynamically.

4.5.1.1 Isothermal Elution

For isothermal, isobaric (constant pressure) elution, measurement of peak retention parameters at several temperatures reveals the previously discussed linear relationship between the reciprocal absolute column temperature T_c (K), and the log of the peaks' retention factors k (the equation is included again here for easy reference)

$$\log(k) = \frac{A}{T_c} + B \tag{4.5}$$

where A and B are arbitrary constants that are specific to individual compounds on a particular column. This relationship is independent of a capillary column's pressure drop because the retention factor k does not depend on it. Thus, Equation 4.5 predicts the retention behavior of peaks whose retention factors are known for at least two temperatures, and variations in pressure drop or flow with column temperature are accommodated by the use of the retention factor, which is independent of the carrier-gas conditions.

It is a simple matter to compute coefficients A and B for each peak of interest by linear regression and then find the peaks' predicted retention factors at specific temperatures, as shown earlier in this chapter. However, the peaks' predicted retention times and not just their retention factors are needed for chromatogram simulation and resolution calculations in the absence of the extensive retention measurements used in the earlier examples. The scenario gets more complex as we combine the effects of temperature, pressure drop, and column dimensions as required for a more complete column system model.

The peaks' retention times are a function of their individual retention factors and the unretained peak time, t_M:

$$t_R = t_M(k + 1) \tag{4.10}$$

Therefore, in addition to knowing the retention factors from Equation 4.5, we will need to predict the unretained peak time at the desired temperatures. Fortunately, if not simply, the unretained peak time is a function of the column dimensions, pressure drop, carrier-gas type, and column temperature: it can be calculated for simulation according to Equation 4.2.

The unretained peak time can be related to the pressure drop, column dimensions, and carrier-gas viscosity by combining the—hopefully—familiar relationship between column length, unretained peak time, and average carrier-gas linear velocity $t_M = L/\overline{u}$ with Equation 4.10 to yield

$$t_M = 32\left[\frac{L}{d_c}\right]^2 \frac{\eta}{\Delta p \cdot j'} \tag{4.11}$$

Interestingly, this equation predicts that at constant temperature and pressure drop, the unretained peak time will not change if the ratio of the column length to the diameter remains constant. A 30-m × 200-μm-i.d. column should have the same unretained peak time as a 15-m, × 100-μm-i.d. column at the same temperature and pressure drop, for example.

The carrier-gas viscosity η increases with rising temperatures according to the following relationship:

$$\eta_T = \eta_0\left(\frac{T}{273.15}\right)x \tag{4.12}$$

Here, η_T is the viscosity at temperature T, η_0 is the viscosity at 273.15 K, and x is an exponent specific to the type of carrier gas (15).

As a bonus, by incorporating the column and carrier-gas variables with the unretained peak time calculation, the simulation can be extended to accommodate dimensional and carrier-gas changes. To do so, however, we first will have to make some changes to Equation 4.5, which is written in terms of the retention factor. It is independent of the carrier gas and pressure drop, but the retention factor does depend on the column dimensions. The peaks' thermodynamic distribution coefficients K remain constant across column dimensional changes, including changes to the stationary-phase film thickness. Rewriting Equation 4.5 with K and changing it to the natural log gives

$$\ln(K) = \frac{b}{T_c} + a \tag{4.13}$$

This is essentially the same as Equation 4.5, except that the new constants a and b are directly related to the enthalpic and entropic thermodynamic coefficients

ΔH and ΔS, respectively

$$a = \frac{\Delta S}{R}$$
$$b = -\frac{\Delta H}{R} \tag{4.14}$$

where R is the universal gas constant. This relationship derives from equilibrium partition theory as well (16), and it is the close correlation of this theory with experimental results that makes accurate gas chromatographic simulations possible.

To use Equation 4.13, we will need to relate the retention factor k to the distribution coefficient. Chromatographers can measure retention factors directly from the chromatogram if the unretained peak time is known. The distribution coefficient K in Equation 4.13, however, is not directly evident, but it can be computed from the measured retention factor, if the column film thickness d_f and inner diameter d_c are known. These two column measurements determine the phase ratio β, which is the ratio of the gas to stationary-phase volumes in the column:

$$\beta \approx \frac{d_c - 4d_f}{4d_f} \tag{4.15}$$

The retention factor and the distribution coefficient are related by the column phase ratio, as shown in this equation:

$$K = \beta \cdot k \tag{4.16}$$

So, if we know the column phase ratio and the peaks' retention factors at various temperatures, we can compute the distribution coefficients, and from there find the thermodynamic parameters that characterize the peaks on a specific stationary phase. Optimization programs utilize at least two, and often three, sets of known retention data at different temperatures in order to assess the thermodynamic variables. Alternatively, by making some assumptions about the value of ΔH, one set of calibration data can be used, but the results will be approximate.

Some optimization system models rely on predetermined libraries of compounds already calibrated on several common stationary phases. If a peak of interest is in such a library and the analyst is using one of the characterized phases, then no additional calibration may be necessary as long as (1) the calibration column and the experimental column dimensions are known with good accuracy, (2) the calibration gas chromatograph's oven temperature and experimental oven temperatures are standardized, and (3) the pressure drops and ambient pressures for the calibration and experimental systems are known accurately. If not, then the simulations will be less accurate. However, small errors in these areas will not distort simulated results so much that peak elution order and relative retention will be meaningless. Even when not exact down to the second, simulations provide a wealth of useful information about peak retention behavior under a range of test conditions.

Now, we are in a position to compute peak retention times in terms of their measured thermodynamic coefficients, the column dimensions, the carrier-gas type, the pressure drop, and the column temperature. We get the following relationship by combining Equations 4.10 and 4.11:

$$t_{R,T} = \left[32(1 + k_T) \cdot \left(\frac{L}{d_c} \right)^2 \right] \times \frac{\eta_T}{\Delta p \cdot j'} \tag{4.17}$$

where the subscripts T refer to the parameters' values at the desired simulated column temperature. The retention factor at column temperature k_T is computed from Equations 4.13, 4.15, and 4.16. Known retention and unretained peak times from calibration runs at two or more temperatures provide the thermodynamic coefficients a and b for this purpose. The viscosity at column temperature comes from Equation 4.12.

Using Equation 4.17, chromatographers can predict the effects of changing column dimensions, linear velocity, phase ratio, carrier gas, pressure drop, and temperature on simulated isothermal retention times for peaks whose retention times are known at as few as two temperatures on a single stationary phase. These manipulations are achievable with a hand calculator and a lot of effort, or more easily with a computer spreadsheet that can also graph the results. Naturally, commercial computer programs excel at these calculations, although they may not all use mathematical expressions that are formulations exactly the same as given here.

Figure 4.14 shows a screen dump of an isothermal gas chromatographic simulation from a commercial gas chromatographic optimization program. The Temperature, Pressure, and Column tabs in the display permit the user to set elution conditions, including multiramp temperature and pressure programming, which were not exercised for this example. The Auto-Optimize tab carries out a minimum-resolution-oriented optimization calculation, which determines a set of conditions that lie within specified limits and meet the minimum resolution criterion.

4.5.1.2 Temperature-Programmed Elution

For programmed temperature (and pressure) elution, most gas chromatographic simulation programs solve Equation 4.9 by transforming the variables from time into distance along the column and then dividing the programmed elution run into a large number of discrete steps at small time increments. Analytical solutions to this integral are not practical, and stepwise approximation lends itself well to computer programs. Instead of computing the retention time, an equation similar to 4.17 is modified to calculate the distance a peak moves along the column in the z direction during each interval. The "motion" of each peak is summed with each step to simulate transport, separation, and elution, as demonstrated earlier in this chapter with the simplified stepwise programming example (17). The transformation of Equation 4.17 from the temperature domain to time can incorporate an arbitrary temperature and pressure program, including

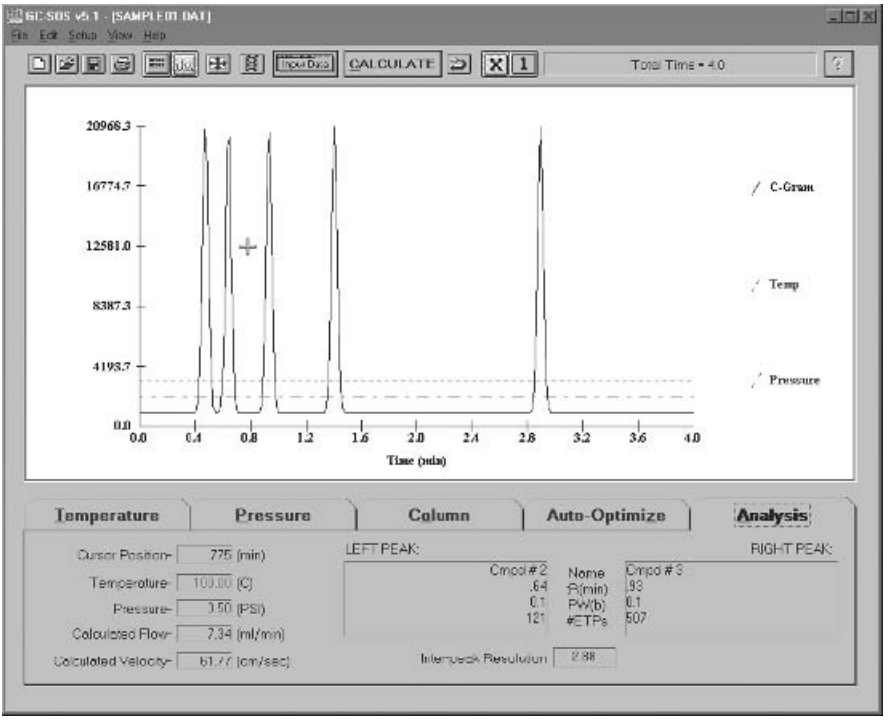


FIGURE 4.14 Example of isothermal gas chromatogram simulation. The Analysis tab (shown) displays the elution conditions plus the retention times, peak widths, effective theoretical plate numbers, and resolution for the two peaks flanking the cursor. Program: GC-SOS v 5.1 (ChemSW, Inc., North Fairfield, CA).

static plateaus and negative ramps (18). Other solutions to the programmed-temperature problem include using a derivative of a liquid chromatography linear elution model (19) and an alternate way of determining thermodynamic data from retention indices (20).

Earlier we simplified the programmed-temperature simulation by assuming that the carrier-gas velocity is constant along the length of the column. Of course, the carrier-gas velocity actually increases from the entrance to the exit of the column, due to carrier-gas expansion. The local carrier-gas linear velocity at any position in the column u_z, together with the retention factor at the local column temperature, determine the distance that a peak moves during one simulation step. When the peak's position equals the column length L, the peak is eluted from the end of the column, and that time is equal to the retention time. If we consider the increments to be infinitely small, then we can write the following integral to express the process of peak elution

$$\int_0^{t_R} z_{i,t}\,dt = L \tag{4.18}$$

where $z_{i,t}$ expresses the position along the column of the ith peak as a function of the time-based temperature and pressure program parameters.

This deceptively simple expression incorporates all the variables mentioned previously, plus temperature and pressure programs as discrete time-based functions. Combining Equations 4.18, 4.17, and 4.13 allows us to express the programmed-temperature elution process in terms of the thermodynamic coefficients, the average carrier-gas linear velocity as a function of the program time, and the column phase ratio:

$$\int_0^{t_R} \left[\frac{\overline{u}}{1 + \frac{a}{\beta} \cdot e^{b/T}} \right] dt = L \tag{4.19}$$

This equation and similar formulations are often used as the basis for commercial gas chromatographic optimization programs. Figure 4.15 illustrates a screen dump of a programmed-temperature simulation from a commercial computer program. Here, one chromatogram from a set of chromatograms that satisfy minimum

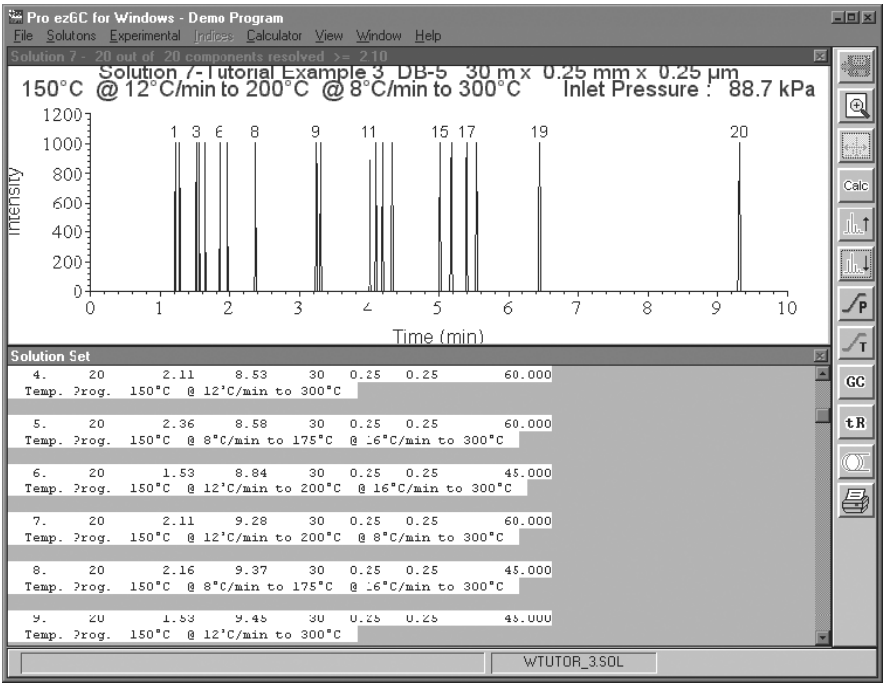

Pro ezGC for Windows - Demo Program
File Solutions Experimental Indices Calculator View Window Help
Solution 7 - 20 out of 20 components resolved >= 2.10
Solution 7 - Tutorial Example 3 DB-5 30 m x 0.25 mm x 0.25 µm
150°C @ 12°C/min to 200°C @ 8°C/min to 300°C Inlet Pressure : 88.7 kPa
Intensity
Time (min)
Solution Set
4. 20 2.11 8.53 30 0.25 0.25 60.000
Temp. Prog. 150°C @ 12°C/min to 300°C
5. 20 2.36 8.58 30 0.25 0.25 60.000
Temp. Prog. 150°C @ 8°C/min to 175°C @ 16°C/min to 300°C
6. 20 1.53 8.84 30 0.25 0.25 45.000
Temp. Prog. 150°C @ 12°C/min to 200°C @ 16°C/min to 300°C
7. 20 2.11 9.28 30 0.25 0.25 60.000
Temp. Prog. 150°C @ 12°C/min to 200°C @ 8°C/min to 300°C
8. 20 2.16 9.37 30 0.25 0.25 45.000
Temp. Prog. 150°C @ 8°C/min to 175°C @ 16°C/min to 300°C
Temp. Prog. 150°C @ 12°C/min to 300°C
Calc
GC
tR
WTUTOR_3.SOL

FIGURE 4.15 Example of temperature-programmed gas chromatogram simulation. The upper window displays the simulated chromatogram and notation. The lower window lists a series of solution sets that meet the user's criteria for minimum resolution of the closest-eluting peaks across a range of elution conditions. Program: Pro ezGC for Windows (Restek Corporation, Bellefonte, PA).

resolution criteria is displayed in the top window, and a synopsis of several of the solution sets appears below it. The program varies the column length, the temperature program, and the pressure drop across defined ranges to arrive at a limited set of acceptable solutions.

A number of second-order effects also come into play, including additional temperature dependencies; compensation for small errors in actual column dimensions, pressures, and temperatures; and mass transport effects that stem from the compressible nature of the carrier gas. For example, during temperature and flow programming the carrier-gas flow through the column is disturbed from its steady-state conditions as the system progresses through changing operating values of temperature and pressure (21). These disturbances propagate through the carrier gas as deviations that the mathematical models presented here do not take into account. One simple approach to such deviations from ideal behavior adds a time-delay factor to the calculations of the peak positions.

4.5.2 Peak Shape Simulation

These mathematical models enable prediction of isothermal or temperature-programmed retention times with very good accuracy, and so chromatographers can estimate the effects of changing conditions on peak elution sufficiently well to provide a good basis for optimization. These models do not take into account any of the band-broadening processes that determine peak shapes, and therefore alone they cannot predict peak resolution, Trennzahl or separation number, or any other measurement of chromatographic quality.

Most simulation programs utilize a simplified approach to this problem and present a stylized chromatogram that only approximates real-world peak shapes as would be obtained from a chromatographic run. Some programs assume that peak widths increase monotonically with increasing isothermal retention time, and base the simulation output on an initial user-provided peak width or on the column inner diameter. Similarly, for a crude approximation programmed-temperature elution can be assumed to produce peaks with a constant width.

Better peak shape simulations take into account the basic van Deemter–Golay equations to compute the degree of peak broadening that would occur under the set of isothermal conditions, or with each simulation step for temperature programming. A more intensive and accurate approach uses the Giddings–Golay equation (22,23), which includes additional compensation terms for carrier-gas expansion. In either case, ultimately the chromatographer must transfer an optimized set of conditions into an instrument and evaluate the efficacy of the optimization procedure.

4.6 CONCLUSION

Optimization of gas chromatography presents analysts with an opportunity to take control of their separations and achieve specific goals. Optimization of

relatively simple isothermal chromatograms with the purpose of incrementally improving speed of analysis or resolution by making small changes in column flowrate, temperature, or length are easily achieved with the data from one or two experiments and some knowledge of the interrelationships of the important variables. Temperature programming introduces additional considerations that can be addressed with a computer, either using general-purpose tools such as spreadsheets or with dedicated chromatography simulation programs. Complex chromatograms in which many peak pairs are just barely resolved are better addressed with computer programs designed for such situations and not by taking guesses about optimum conditions. In all cases, the data that form the basis for making optimization decisions must be accurate, and the analyst must be sure to verify the efficacy of the suggestions of an optimization routine. A thorough understanding of the chromatographic principles that lie behind separations is invaluable.

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CHAPTER FIVE

High-Speed Gas Chromatography

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PART 1 OVERVIEW

5.1 CAPILLARY COLUMNS FOR HIGH-SPEED GC (HSGC)

Shortly after the introduction of the wall-coated open-tubular column in 1957 (1), it was recognized that speed could be substituted for resolution in a much more favorable way than for packed columns. The reason is the openness of the wall-coated column (greater gas permeability) results in a substantially smaller pressure drop per length of column. Two options became apparent. Much longer columns could be used relative to packed columns to increasing resolving power, or higher flow rates could be used with shorter columns to achieve faster separations.

The use of longer columns for the analysis of more complex mixtures than can be handled by packed columns has been profoundly developed. Open tubular columns with 30–60 m lengths are used routinely. The other option has received much less attention until quite recently. This chapter will consider the theory, instrumentation and separation strategies needed for obtaining order-of-magnitude or greater reductions in gas chromatographic analysis time and the application of high-speed separation methods to increasingly complex mixtures.

5.2 NEED FOR HIGH-SPEED SEPARATIONS

It is generally agreed that GC and GCMS are the most widely used methods for the analysis and characterization of mixture of volatile and semivolatile organic compounds. Industry experts estimate the number of GC instruments in routine

use worldwide in the range 0.25–0.35 million. Increasing demands for higher sample throughput are beginning to focus more attention on the development of high-speed GC (HSGC) systems.

These developments have resulted in several new technologies for HSGC. These technologies include microbore ($\leq$0.1-mm-i.d.) columns, new methods for very fast column heating, inlet devices that inject very narrow sample plugs, dual-column methods for enhancing selectivity, and time-of-flight mass spectrometry for high-speed mixture characterization. In addition, evolutionary changes in GC instruments from major manufacturers are allowing for convenient retrofitting with the new technologies. These technologies will be discussed in this chapter.

5.3 PIONEERING STUDIES

In 1962, Desty et al. (2) described a system for HSGC that used a 2-m-long, 0.07-mm-i.d. open tubular column, a flame ionization detector and a split injector to separate the nine heptane isomers in 5 s. In order to obtain a sufficiently narrow plug of vapor at the column inlet, a dilute vapor sample was used, and the syringe plunger was struck with a mallet. High-speed chromatograms were recorded on photographic film using a galvanometer with a rotating mirror. Despite these early successes, only a few studies were reported until the late 1970s.

Jonker et al. (3) in 1962 used short packed columns with very small support particles and high inlet pressures to demonstrate very fast separations. The introduction of the flexible, fused-silica open tubular column in 1979 (4) lead to the gradual acceptance of the wall-coated, open tubular column as a very general high-performance separation tool. Throughout the 1980s, microbore fused-silica columns gained popularity, particularly in Europe, as a means for reducing analysis time (5).

In 1983, Angell et al. (6) described a microfabricated GC using a channel etched in a silicon chip as a column. Valve seats, sample loop, and thermal conductivity detectors were also micro-fabricated on the chip. Column performance was relatively poor, and eventually the column was removed from the chip and replaced with a fused-silica microbore column. This led to commercial portable instruments for HSGC (7).

As early as 1958, Dal Nogare and Bennett (8) used resistive heating of a stainless-steel packed column to achieve high-speed temperature programming with rates as high as 200°C/min. Since the late 1980s, practical approaches to high-speed temperature programming of fused-silica columns have been described (9). In the late 1970s and through the 1980s, several studies showed that operating a GC column at reduced outlet pressure (vacuum outlet GC) could substantially reduce analysis time particularly for very fast separations using relatively short, wide-bore open tubular columns (10,11).

5.4 LITERATURE ON HIGH-SPEED GC

Literature for HSGC is widely scattered through several journals, a few reviews of HSGC, and some reviews that consider topics related to HSGC. The most

useful journals include *Analytical Chemistry*, *High-Resolution Chromatography* (now *Journal of Separation Science*), *Journal of Chromatography Part* A and *Chromatographia*.

Sacks et al. have considered several emerging technologies for HSGC (12) and tunable column selectivity for HSGC and GCMS (13). In a comprehensive study, Hinshaw and Ettre (14,15) discuss the use of tandem columns with pressure-tunable selectivity. Sandra et al. (16) have reviewed the role of GC column selectivity and the means for controlling selectivity. Papers considering high-speed temperature programming (17,18), inlet systems (19–21), and vacuum outlet GC (10,11) should be consulted. Extracolumn band broadening in HSGC has been discussed in detail (22). Finally, a special issue of *High Resolution Chromatography*, which featured comprehensive two-dimensional gas chromatography (2DGC), contains considerable information on instrumentation and issues related to HSGC (23).

PART 2 COLUMN DESIGN AND OPERATING CONDITIONS

5.5 REQUIREMENTS FOR HIGH-SPEED GC

5.5.1 Column Length, Carrier-Gas Flowrate, and Temperature

Analysis time for the separation part of any analytical method is defined by the retention time t_{Rl} for the last target component peak to elute from the column.

$$t_{\mathrm{Rl}} = \frac{L}{u}(k_l + 1) \tag{5.1}$$

where u is the average carrier-gas velocity, L is the column length and k_l is the retention factor for the last compound. If the column length used for an analysis is reduced by a factor of 5 and the carrier-gas velocity increased by a factor of 4, the analysis time is reduced by a factor of 20. For example, a 6-m-long column operated with hydrogen at an average velocity of 200 cm/s has a holdup time of 3.0 s, and the analysis time for the isothermal analysis of a mixture with a retention factor range of 0–10 is 33 s.

Retention factors are related to column temperature T_{c} by

$$\ln k = \frac{A}{T_{\mathrm{c}}} + B \tag{5.2}$$

where A and B are constants that are unique for every compound and for every stationary-phase type and phase volume ratio. Plots of $\ln k$ versus $1/T_{\mathrm{c}}$ are known as *van't Hoff plots*. These plots are reasonably linear over a limited temperature range and are very useful for addressing the effects of column temperature and temperature program on analysis time and column selectivity. Note that retention

is very sensitive to temperature, and typically a 15–20°C increase in column temperature will result in a twofold reduction in solute retention factors.

5.5.2 Problems with HSGC

There are two major difficulties for the successful use of HSGC:

1. Conventional GC instruments are inadequate. When short capillary columns are used at high flowrates, the effects of extracolumn sources of band broadening are amplified, and injection plug width, detection method, and data-processing techniques may contribute to large reductions in system resolving power.
2. Peak capacity n_p (number of perfectly spaced peaks that will fit in a chromatogram with a specified resolution R_s) is reduced with shorter columns as described by

$$n_p = 1 + \frac{\sqrt{L/H}}{4R_s} \ln\left(\frac{t_{Rl}}{t_M}\right) \tag{5.3}$$

where t_M is the holdup time for the column and H is the height equivalent to a theoretical plate for the column. Thus, if separation time is reduced by a factor of 4 by a corresponding reduction in column length, the peak capacity is reduced by a factor of 2. This loss in peak capacity makes it more difficult to apply HSGC methods to more complex mixtures.

5.6 COLUMN EFFICIENCY AND OPERATING CONDITIONS

The use of short columns and high flowrates results in significant losses in column resolving power. The result is increased probability of peak overlap. This amplifies the importance of using conditions that provide the maximum possible column efficiency. Open tubular columns used for HSGC usually have relatively thin stationary-phase films. If the ratio of column diameter to film thickness is equal to or greater than about 1000, band broadening in the stationary phase for polysiloxane and poly(ethylene glycol) (PEG) phases often can be neglected relative to other sources of band broadening. Only thin-film columns are considered in this section. The effects of carrier-gas type, flowrate, column length, and column diameter on column efficiency are considered.

Differentiation of the Golay equation (1) leads to Equations 5.4 and 5.5 for the minimum height equivalent to a theoretical plate H_{min} and the optimal average carrier-gas velocity needed to achieve the minimum plate height u_{opt}.

$$H_{min} = r\sqrt{\frac{1 + 6k + 11k^2}{3(k+1)^2}} \tag{5.4}$$

$$u_{opt} = \frac{jD_G}{r}\sqrt{\frac{48(k+1)^2}{1 + 6k + 11k^2}} \tag{5.5}$$

where r is the column radius, D_G is the binary diffusion coefficient of the solute in the carrier gas, k is the solute retention factor, and j is the Martin–James gas compressibility correction (24) for the column inlet and outlet pressures p_i and p_o, respectively:

$$j = \frac{3(P^2 - 1)}{2(P^3 - 1)} \tag{5.6}$$

$$P = \frac{p_i}{p_o} \tag{5.7}$$

From Equation 5.4 it is clear that smaller column radius favors greater column efficiency (smaller plate height), and from Equation 5.5 that small column radius and large binary diffusion coefficients favor high optimal carrier gas velocity, both of which are desirable for HSGC.

5.6.1 Carrier Gas and Column Dimensions

Figure 5.1 shows a comparison of hydrogen, helium, and air as carrier gas for a 10-m-long, 0.25-mm-i.d. thin-film column assuming a retention factor of 2.0. Literature values of carrier-gas viscosity and of D_G for benzene at 50°C were used. Note that the viscosity of helium and air are quite similar, and the differences in the plots for these carrier gases are due primarily to the differences in the diffusion coefficients. On the other hand, the diffusion coefficients for helium and hydrogen are more similar, and the differences in the plots for these gases are due largely to the differences in their viscosities, which result in different values of j for any specified carrier-gas velocity.

Note from Equation 5.4 that H_{min} is independent of the carrier gas. This is seen in Figure 5.1. From Equation 5.5, the value of u_{opt} is proportional to jD_G. The combination of small D_G and large viscosity (small j) results in a small u_{opt} for air relative to the other gases. Hydrogen has a larger u_{opt} than helium, but the difference is relatively small. For high carrier-gas velocity values and for thin-film columns, H is approximated by

$$H = \frac{1 + 6k + 11k^2}{24(k + 1)^2} \frac{r^2}{jD_G} u \tag{5.8}$$

The coefficient of u in this equation gives the local slope of the right-hand flank of the Golay plots in Figure 5.1. Note that the slope is inversely proportional to jD_G. This explains why efficiency with helium as carrier gas decreases more rapidly with increasing u than with hydrogen. Thus, hydrogen become progressively more useful as a carrier gas for HSGC as the gas velocity increases. Air and nitrogen are particularly poor for HSGC, since u_{opt} is low and significant departure from u_{opt} results in a large loss in efficiency.

Figure 5.2 shows plots of H versus u for 10-m-long, thin-film columns using hydrogen carrier gas at 50°C and assuming a retention factor of 2.0. A D_G value of 0.4 cm^2/s was used for all plots. Plots are shown for column diameters of

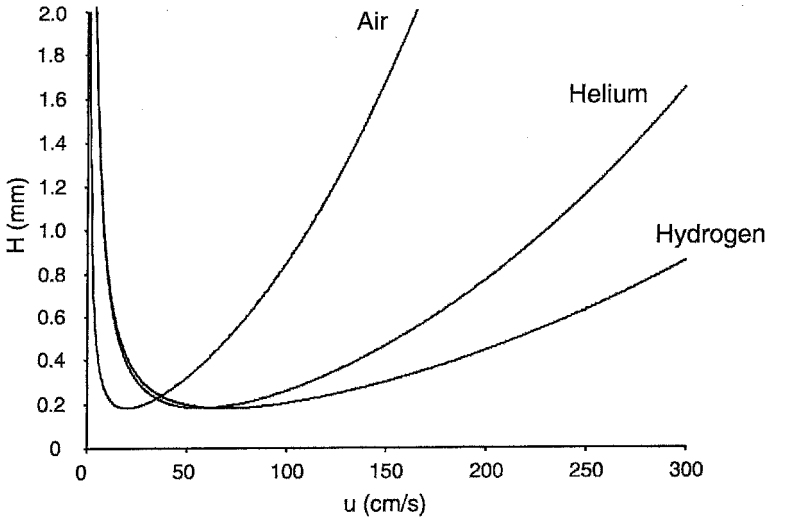

FIGURE 5.1 Golay plots for air, hydrogen, and helium as carrier gases using a 10-m-long, 0.25-mm-i.d. thin-film column. Literature values of gas viscosity and binary diffusion coefficients for benzene at 50°C are used. A retention factor of 2.0 is assumed.

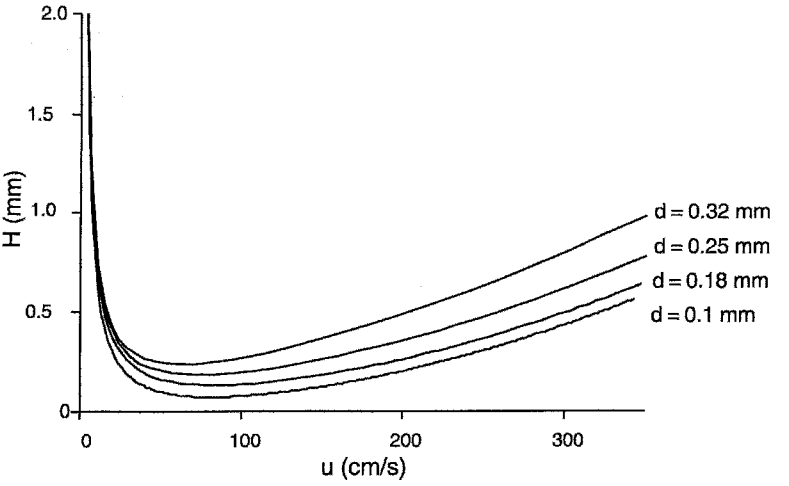

FIGURE 5.2 Golay plots for 10-m-long, thin-film columns of various diameters using hydrogen as carrier gas. A binary diffusion coefficient of 0.4 cm^2/s and a retention factor of 2.0 are assumed.

0.32, 0.25, 0.18, and 0.10 mm. The values of H_{min} decrease, and the values of u_{opt} increase with decreasing column radius, and substantially faster separations can be obtained with the microbore (0.1-mm-i.d.) column.

The value of H_{min} is independent of gas compression effects, and the decrease in H_{min} with decreasing column diameter, which is clearly seen in the figure,

is proportional to the decrease in column radius (see Equation 5.4). The shift in u_{opt} to larger values with decreasing column diameter does vary with j, and the shift is smaller for longer columns, which require a higher inlet pressure (smaller j) for any specified value of u. Also, the shape of the right-hand flank of the plots varies with j, and again this degrades the performance of the columns with smaller diameter more than for the columns with larger diameter. Thus, the advantages of larger u_{opt} and reduced loss in efficiency for large u values are greatest for relatively short microbore columns with hydrogen as carrier gas, since this results in a smaller value of P and thus larger j.

The downside of small column diameter is that for a given film thickness there is less stationary phase per unit length, and thus the amount of sample that can be injected without stationary-phase overloading is significantly reduced. Also, to minimize the effects of band broadening in the stationary phase, thinner stationary-phase films often are used for HSGC. These factors can result in detection limitations for trace analysis. Another limitation of small-diameter columns is that their volumetric flowrates can be very low. This can result in significant peak broadening with some inlets and detectors.

It is frequently observed that shorter columns are more efficient than longer ones at higher flowrates. This is explained entirely by gas compression effects. A decrease in j caused by an increase in inlet pressure associated with longer columns results in a shift in u_{opt} to smaller values. This is seen in Figure 5.3 for various lengths of 0.20-mm-i.d. columns using hydrogen as carrier gas at 50°C with k and D_G values of 2.0 and 0.4 cm^2/s, respectively. In addition to a shift

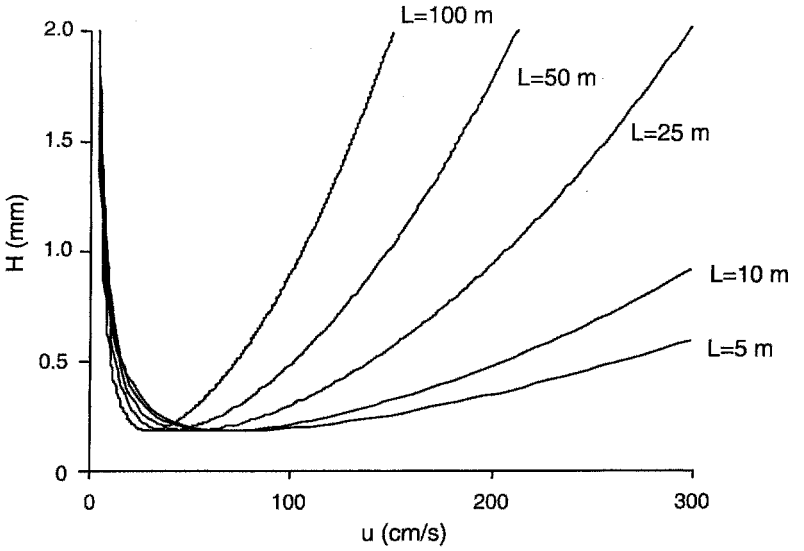

FIGURE 5.3 Golay plots for 0.20-mm-i.d thin-film columns of various lengths using hydrogen as carrier gas. A binary diffusion coefficient of 0.4 cm^2/s and a retention factor of 2.0 are assumed.

to lower u_{opt} values, the slope of the right-hand flank of the plots in Figure 5.3 is larger for longer columns. This results in substantially greater efficiency for shorter columns when operated at the relatively high average carrier-gas velocities used for HSGC. However, the total number of theoretical plates and thus the column resolving power decreases steadily with decreasing column length.

5.6.2 Vacuum-Outlet GC

Column efficiency at high average carrier-gas velocities can be increased by operating the column at reduced outlet pressure. Gas-phase binary diffusion coefficients scale inversely with gas density and thus any reduction in pressure results in larger D_G values. This causes an increase in longitudinal diffusion and a decrease in resistance to mass transport in the gas phase. The result is an increase in u_{opt} and a decrease in the slope of the right-hand flank of the Golay plots (see Equations 5.5 and 5.8).

The D_G values used in Equations 5.5 and 5.8 are specified for the column outlet pressure. When a vacuum pump is used to reduce the column outlet pressure, the D_G values at the outlet pressure are found as the product of the atmospheric-pressure values and the ratio of atmospheric pressure to the column outlet pressure. Figure 5.4 shows column efficiency (Golay) plots for 5-m-long, thin-film columns operated with hydrogen carrier gas at 50°C and assuming a D_G value of 0.4 cm^2/s at atmospheric pressure. Broken-line plots are for an outlet pressure of 1.0 atm, and the solid-line plots are for an outlet pressure of 0.01 atmosphere. The top pair of plots is for a 0.1-mm-i.d. column, the center pair for a 0.25-mm-i.d. column, and the bottom pair for a 0.53-mm-i.d. (megabore) column.

For the 0.53-mm-i.d. column, u_{opt} shifts from less than 30 cm/s for the atmospheric-pressure case to over 250 cm/s for the vacuum outlet case. In addition, the right-hand portion of the plot for the vacuum-outlet case is very flat, and little loss in efficiency occurs for u values over 350 cm/s. This allows for much faster separations. The advantage of vacuum outlet operation diminishes with decreasing column diameter, and for the 0.1-mm-i.d. column, it is relatively small. The speed advantage for vacuum outlet GC increases as the inlet pressure decreases (larger D_G values). Since the inlet pressure required to achieve a specified value of u increases with increasing column length and with decreasing column diameter, vacuum outlet techniques are most useful for very fast separations with relatively short and wide-bore columns. However, resolving power is relatively low for these columns.

PART 3 INSTRUMENTATION

5.7 INSTRUMENTAL REQUIREMENTS FOR HSGC

5.7.1 Sources of Extracolumn Band Broadening

In addition to the solute band broadening that occurs on the column, band broadening occurs from other processes. Major sources of extracolumn band

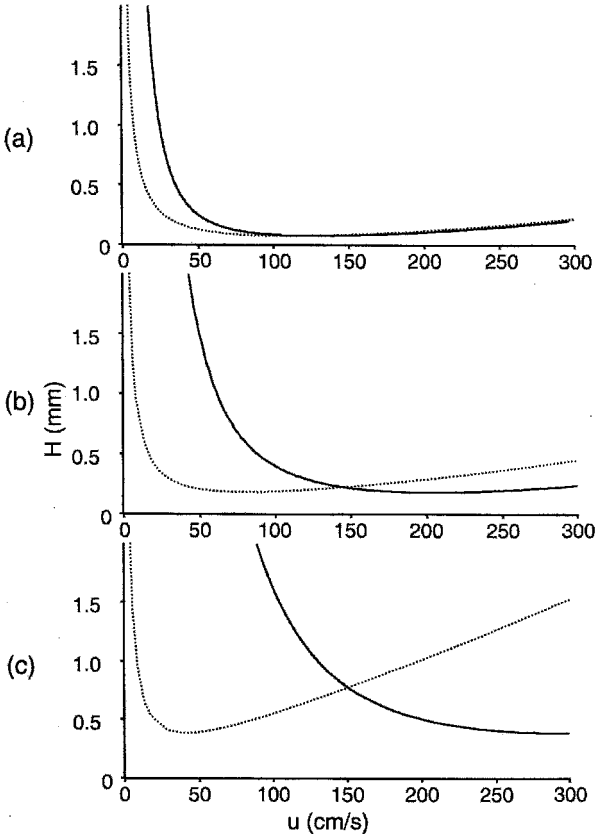

FIGURE 5.4 Golay plots for atmospheric outlet pressure (dotted lines) and 0.01 atmosphere outlet pressure (solid lines) using 5.0-m-long columns with hydrogen carrier gas and i.d. values of 0.10 mm (a), 0.25 mm (b), and 0.53 mm (c). A binary diffusion coefficient of 0.4 cm^2/s and a retention factor of 2.0 are assumed.

broadening include the injector, the detectors, the plumbing connecting the injector to the column and the column to the detector, and the data system. For conventional (slow) GC, on-column band broadening usually is large enough to mask extracolumn band broadening, and the latter often is not considered in calculations of column resolving power. For HSGC, on-column band broadening may be reduced, while extracolumn band broadening may become very significant, particularly for a poorly designed gas chromatographic instrument. The peak variance for extracolumn band broadening σ_{ec}^2 is given by the equation (22)

$$\sigma_{ec}^2 = \frac{\Delta t^2 u^2}{(k+1)^2} \tag{5.9}$$

where Δt is the total instrumental dead time. Division of Equation 5.9 by the column length gives the contribution to the plate height H_{ec} from

extracolumn processes:

$$H_{ec} = \frac{\Delta t^2 u^2}{L(k+1)^2} \tag{5.10}$$

5.7.2 Column Efficiency with Extracolumn Band Broadening

When Equation 5.10 is compared with Equation 5.1 for component retention time, it is seen that the relatively short columns, high flowrates and small k values needed to reduce analysis time all result in increased plate height from extracolumn band broadening. Note in particular the u^2 dependence. For example, if the column length is reduced by a factor of 5 and the average carrier-gas velocity increased by a factor of 4, the analysis time is reduced by a factor of 20, but the contribution from extracolumn band broadening to overall plate height is increased by a factor of $4^2 \times 5 = 80$. A comparable reduction in extracolumn band broadening can be obtained by reducing Δt in Equation 5.10 by a factor of $80^{1/2} = 8.9$.

For HSGC with thin-film columns and high flowrates, Equations 5.8 and 5.10 are combined to give Equation 5.11 for plate height considering extracolumn band broadening:

$$H = \frac{1 + 6k + 11k^2}{24(k+1)^2} \frac{r^2}{j D_G} u + \frac{\Delta t^2}{L(1+k)^2} u^2 \tag{5.11}$$

Note that when extracolumn band broadening is neglected, the right-hand flank of Golay plot (high average carrier-gas velocity) is a nearly linear function of u, but with extracolumn band broadening, the plots become quadratic in u. This is clearly seen in the Golay plots in Figure 5.5 for a 5-m-long, 0.25-mm-i.d. thin-film column using hydrogen as carrier gas with $k = 2.0$ and $D_G = 0.4$ cm^2/s. Values of Δt for the plots range from 0 to 1.0 s. For a Δt value of 0.01 s, extracolumn band broadening is insignificant. For Δt values greater than 0.1 s, the loss in column efficiency is very large, particularly for the high average carrier-gas velocities used for HSGC. To reduce instrumental dead time to values substantially less than 0.05 s requires consideration of the entire gas chromatographic instrument.

5.8 INLET SYSTEMS FOR HSGC

In most HSGC work, extracolumn band broadening is dominated by sample injection. A manual injection using a conventional inlet with a splitter has a Δt typically in the range 0.05–0.1 s. As seen in Figure 5.5, this can result in a large loss of column efficiency at high carrier-gas velocities. Inlet systems suitable for HSGC are described in this section.

5.8.1 Valves and Sample Loops

Mechanical valves are useful for injecting narrow vapor plugs into a capillary column. With low dead-volume multiport valves equipped with sample loops,

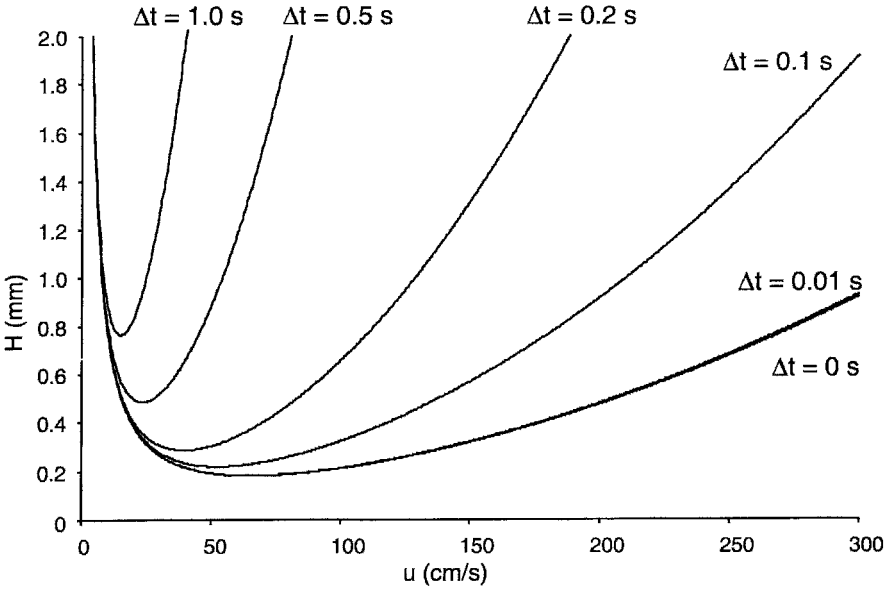

FIGURE 5.5 Golay plots for a 5.0-m-long, 0.25-mm-i.d. thin-film column using hydrogen carrier gas showing the effects of extracolumn band broadening defined by the total instrumental dead time Δt. A binary diffusion coefficient of 0.4 cm^2/s and a retention factor of 2.0 are assumed.

injection plug widths of a few ms can be obtained. For example, a 5-μL sample loop would produce a plug width of about 60 ms for a column flow rate of 5.0 mL/min. A mechanical valve with a sample loop has been used in place of a thermal modulator for comprehensive 2DGC (see below) (25). The actuation of the valve also provides a good zero-time marker for the reliable measurement of retention times.

A microfabricated valve with a sample loop, actuated by an electromagnet, is used in some portable GC instruments (7). Usually these instruments use a microfabricated thermal conductivity detector and a microbore column. The use of this approach, however, is limited, since the volume of injected vapor can be changed only by changing the volume of the sample loop.

The mechanical valve device shown in Figure 5.6 provides for computer-controlled injection plug width (26). Sample vapor is delivered continuously from a small orifice in the side of the sample delivery tube. A variable-speed motor or a stepper motor is used to translate the sample delivery tube so that the orifice passes the end of the column. The width of the injected sample plug is controlled by the velocity of the sample delivery tube. Only when the orifice is aligned with the end of the column will sample vapor be delivered to the column. Using a microstepper motor to move the sample delivery tube, very reproducible vapor plugs can be obtained with a wide range of plug widths. Injection plug widths of less than 10 ms have been reported. The very fast

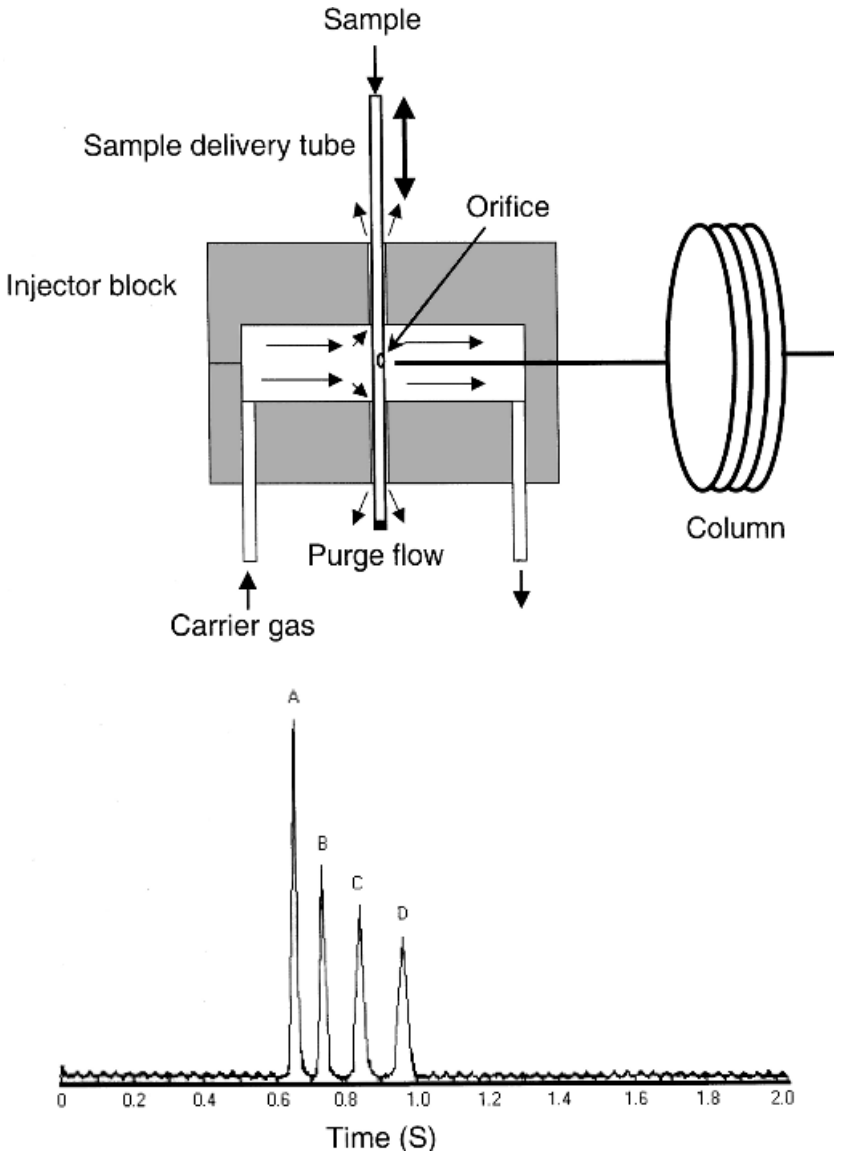

FIGURE 5.6 Gas valve inlet system for HSGC. The orifice in the side of the sample delivery tube is swept past the end of the column to inject a narrow vapor plug. Between injections, the orifice is positioned so that purge flows of carrier gas can prevent sample from entering the column. The chromatogram was obtained with a 2.0-m-long, 0.25-mm-i.d. thin-film dimethylpolysiloxane column with an average carrier-gas velocity of 300 cm/s. Compounds are A, methane; B, dichloromethane; C, trichloromethane; D, tetrachloromethane.

chromatogram show in Figure 5.6 was obtained using a 2.0-m-long, 0.25-mm-i.d. column with an average carrier gas velocity of 300 cm/s and an injection plug width of about 10 ms.

5.8.2 Electrically Heated Metal Cold Traps

A limitation of valve devices is the small quantity of injected sample. Several types of preconcentration devices have been designed to collect volatile and semivolatile compounds from large-volume vapor sample and inject the compounds into the gas chromatographic column as narrow vapor plugs. Figure 5.7 shows cryofocusing inlet systems for HSGC. Both configurations use a bare metal capillary trap that is cooled by a flow of cold gas during sample collection, and rapidly heated by means of a current pulse through the tube in order to inject the sample. For sample collection, the trap tube is cooled to -50 to -100°C. Trapped compounds slowly creep through the cold-trap tube at rates that depend on the component vapor pressure at the trapping temperature and the gas flowrate through the trap. Eventually, each compound breaks through the downstream end of the trap tube, and this sets the upper limit on sample collection time. However, breakthrough times for low-temperature traps may be large.

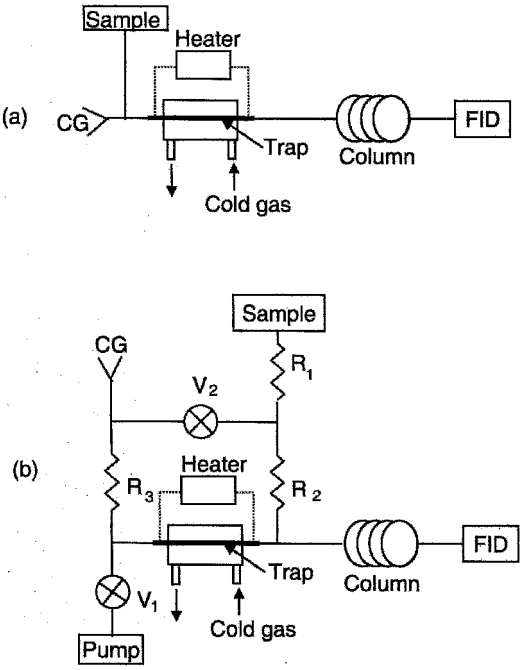

FIGURE 5.7 Cryofocusing inlet systems for HSGC. Carrier gas is supplied at CG. For configuration (b), the carrier gas flow direction is reversed prior to heating the trap for sample vapor injection.

High-boiling point compounds may be trapped near the upstream end of the trap tube. For configuration (a), in Figure 5.7, when the trap tube is heated, the resulting vapor plug must travel through the hot metal tube, and this can cause sample decomposition. In addition, the trap tube may contribute significant dead volume to the injection process resulting in increased extracolumn band broadening.

These problems are nearly eliminated with configuration (b), which uses a valve system (V_1 and V_2) and a vacuum pump to control the gas-flow direction through the trap tube (20). Capillary restrictors R (segments of deactivated fused-silica tubing) are used to control flowrates and pressure drops in the system. Note that the valves are not in the sample flow path, and need not be in the GC oven. For sample collection, V_1 is open and V_2 is closed. Sample vapor is pulled through R_1 and R_2 and passes through the cold-trap tube from right to left. Organic compounds are condensed on the cold metal surface as a narrow, focused plug along the temperature gradient at the right-hand end of the trap tube. A steep temperature gradient favors the formation of a narrow condensed-phase sample plug.

After sample collection is complete, V_2 is opened, and carrier-gas flow through R_1 and R_2 purges these lines and completes the sampling process. Next V_1 is closed, and the trap tube is pressurized with carrier gas through R_3. This reverses the flow direction through the trap tube. The tube is then heated by the current pulse from a capacitive discharge power supply to inject a vapor plug 5–10 ms in width. This inlet system has been used for the direct high-speed analysis of airborne organic compounds (27) and as the trapping part of a high-speed purge-and-trap device (28). An example is shown in Figure 5.8. Since organic compounds can be collected and focused from a large-volume sample, low detection limits can be obtained.

5.8.3 Phase-Coated Thermal Modulators

If a segment of a coated gas chromatographic column is operated at a lower temperature than the oven, or if the stationary phase film is thicker in that portion of the column, the increased retention factors for mixture components in the segment result in reduced migration velocities, and thus sample accumulation occurs in the segment. If the segment is then rapidly heated, the accumulated sample enters the separation column in the GC oven as a relatively narrow band. If a gas stream containing organic compounds with constant or slowly changing concentration passes through the column segment, and if the segment is heated and cooled repeatedly, the sample concentration entering the gas chromatographic column is modulated at the heating cycle frequency. This device is known as a *phase-coated thermal modulator* (21).

The design of thermal modulation devices is undergoing rapid development because of their use in comprehensive two-dimensional GC (2DGC). Figure 5.9 illustrates some useful designs. Design (a) relies on a heater that moves along the thermal modulator column at a controlled speed in order to accelerate the band of

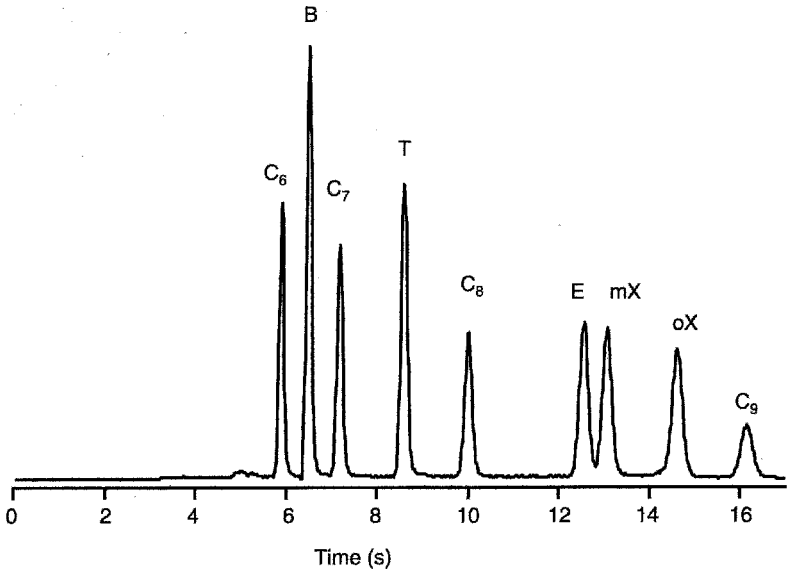

FIGURE 5.8 High-speed chromatogram obtained with the cryo-focusing inlet of Figure 5.7(b). The airborne sample contained C6–C9 *n*-alkanes and benzene (B), toluene (T), ethylbenzene (E), *m*-xylene (mX), and *o*-xylene (oX).

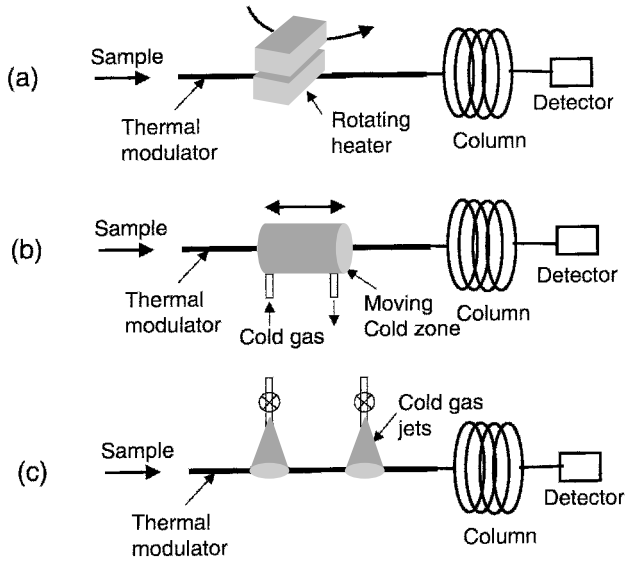

FIGURE 5.9 Phase-coated thermal modulators using a rotating heater (a), moving cold zone (b), and cold gas jets (c) to modulate the concentration of the sample vapor entering the device. For configuration (c), the jets are turned on and off alternately to modulate the sample vapor concentration.

accumulated sample toward the inlet of the column. Alternatively, moving cold zones (b) and valves that control the flow of cold gas directed at the modulator (c) are used to modulate the flow of sample into the column. A thermal modulator can be very simple, and with rapid heating of the accumulated sample, injection plugs of 10–50 ms are easily obtained.

5.8.4 Sorption Traps

Current trends in gas chromatographic instrumentation include the development of smaller, more autonomous (low- or zero-maintenance) instruments. Completely microfabricated instruments are on the horizon. These instruments will be particularly attractive for environmental monitoring. Sorption-based traps are being developed for these instruments. A sorption trap is similar to a phase-coated thermal modulator except the stationary phase is replaced with a solid coating or packing that has a high affinity for the target compounds. Simplicity and potential application to very volatile compounds are important advantages. However, very narrow injection plugs, which are so useful for HSGC, may be more difficult to obtain.

Figure 5.10 shows a trap design and a chromatogram obtained using the device. The device was designed to collect a wide range of organic compounds from large-volume air samples. During sample collection, flow is from left to right, and during sample injection, the flow is from right to left. The device uses three different commercial adsorbent materials. Beds A and B are made from Carbopack Y and Carbopack X, respectively, which are graphitized carbon blacks. Bed A is the weakest adsorber, and this bed strips the highest-boiling-point components from the air sample. Bed B is of intermediate strength and thus removes compounds of intermediate volatility. Bed C contains Carboxen 1000, which is a very large-surface-area molecular sieve material capable of stripping volatile compounds such as acetone, isopropyl alcohol, 2-butanone, and ethyl acetate from large-volume air samples. Since the flow direction is reversed for sample injection, the higher-boiling-point compounds never come in contact with bed C from which they would be difficult to desorb.

5.9 DETECTORS FOR HSGC

When relatively short capillary columns are operated at unusually high flowrates, fast separations are obtained, and peaks are significantly narrower than with conventional GC. To take full advantage of the resolving power available with HSGC, fast detectors and data systems are required. Most laboratory HSGC has been done with flame ionization detection (FID), and most on-site monitoring with portable instruments has been done with thermal conductivity detection (TCD) or FID.

The use of TCD for portable instruments is attractive because the TCD is easily microfabricated (6). Commercial devices have internal volumes of less

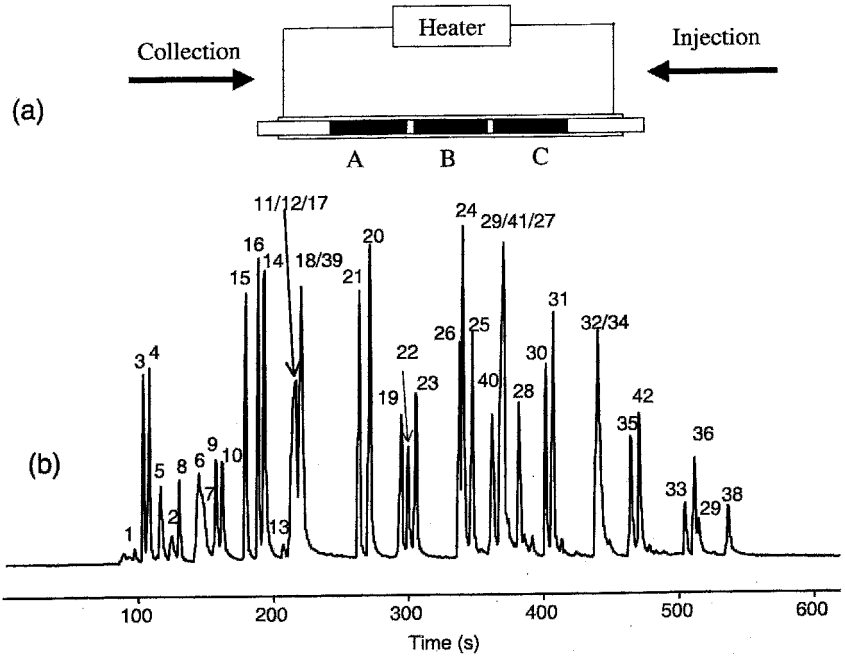

FIGURE 5.10 Reverse-flow sorption trap (a) and the chromatogram of a 42-component mixture in an air sample collected with the device (b). The three adsorption beds are graded; bed A is the weakest absorber and C is the strongest (largest surface area) adsorber. The flow direction is reversed between sample collection and injection. Compounds are 1, acetaldehyde; 2, methyl alcohol; 3, *n*-pentane; 4, isoprene; 5, acetone; 6, ethyl alcohol; 7, 2-propyl alcohol; 8, *n*-hexane; 9, butanone; 10, ethylacetate; 11, 1-propyl alcohol; 12, 2-butyl alcohol; 13, trichloromethane; 14, benzene; 15, isooctane; 16, *n*-heptane; 17, 2-pentanone; 18, 2,5-dimethylfuran; 19, 1-butylalcohol; 20, toluene; 21, *n*-octane; 22, hexanal; 23, butylacetate; 24, ethylbenzene; 25, *m*-xylene; 26, *n*-nonane; 27, *o*-xylene; 28, cumene; 29, α-pinene; 30, β-pinene; 31, *n*-decane; 32, 1,2,4-trimethylbenzene; 33, benzaldehyde; 34, *d*-limonene; 35, 1,2,3-trimethylbenzene; 36, 1,2-dichlorobenzene; 38, *n*-dodecane; 39, 3-pentanone; 40, 1-pentyl alcohol; 41, 2-heptanone; 42, *n*-undecane.

than 1 μL and usually contribute no significant extracolumn peak broadening. Drawbacks of the TCD for environmental applications include relatively poor detection limits and sensitivity to fixed gases and water vapor.

5.9.1 Open-Cell Flame Detectors

Open-cell, flame-based detectors typically have response times of a few milliseconds or less if the capillary separation column is passed through the burner tip and positioned just below the base of the flame. Thus, Δt is small, and σ^2_{ec} from the detector usually is negligible relative to other extracolumn sources of band broadening. While many HSGC studies have used the FID, few data are

available for other flame-based detectors such as the flame thermionic detector and the flame photometric detector. These detectors should be suitable for HSGC.

5.9.2 Closed-Cell Detectors

For closed-cell detectors, including the photoionization detector, the electron-capture detector and the TCD, extracolumn band broadening can be excessive unless specially designed devices with small cell volume are used or the detector is operated at subambient pressure. At reduced column outlet pressure, the carrier-gas velocity in the detector is increased, and the cell is swept out more quickly. Extra gas, called *makeup gas*, can be introduced into the detector cell to sweep the cell more rapidly and reduce peak broadening and distortion.

Microfabricated sensors, which show promise as detectors for HSGC, usually are closed-cell devices, but with very small internal volume. Several devices for sensing organic vapors have been described. Promising sensing methods include the use of surface acoustic wave (SAW) devices (29) and chemiresistor devices (30). Both devices are based on organic vapors partitioning into a thin coating (sensing element) deposited on a set of interdigital (closely spaced but not touching) metal electrodes deposited on a substrate surface. For the SAW sensor, the film is a polymer that is part of a resonance circuit, and the resonance frequency changes because of the change in the film mass when a solute partitions into the film. The shift in frequency is related to the mass of sample that partitions into the film. The chemiresistor device uses a spray-coated layer of gold nanoparticles (5–10 nm diameter) with a self-assembled monolayer of an organic gold thiol. The resistance of the sensing element increases, usually because of swelling, when the solute partitions into the sensing element.

Figure 5.11 shows chromatograms obtained with a SAW sensor (a) and a chemiresistor detector (b). The microfabricated SAW device was made at Sandia National Laboratory and uses a 65-nm-thick film of polyisobutylene as a sensing element. The chemiresistor uses a monolayer of *n*-C8 on the surface of the gold particles as a sensing element. Both sensors have cell volumes of about 5 μL. Both chromatograms were obtained with vacuum outlet GC using atmospheric-pressure air as carrier gas. For an outlet column flow rate of about 5 mL/min, the detector cell sweep time is about 60 ms, which is acceptable for the peak widths observed in the chromatograms. Here, the combination of small cell volume and the increased local carrier-gas velocity in the detector due to the subambient pressure in the cell results in relatively small extracolumn band broadening and obviates the need for makeup gas.

5.9.3 Time-of-Flight Mass Spectrometers

For the characterization (compound identification) of the bands eluting from a fast gas chromatographic separation, time-of-flight mass spectrometry (TOFMS) is unsurpassed. The two unique attributes of TOFMS that make it so well suited as a detector for HSGC are very high spectral acquisition rates and spectral continuity across the chromatographic peak profile for a single-component peak (31).

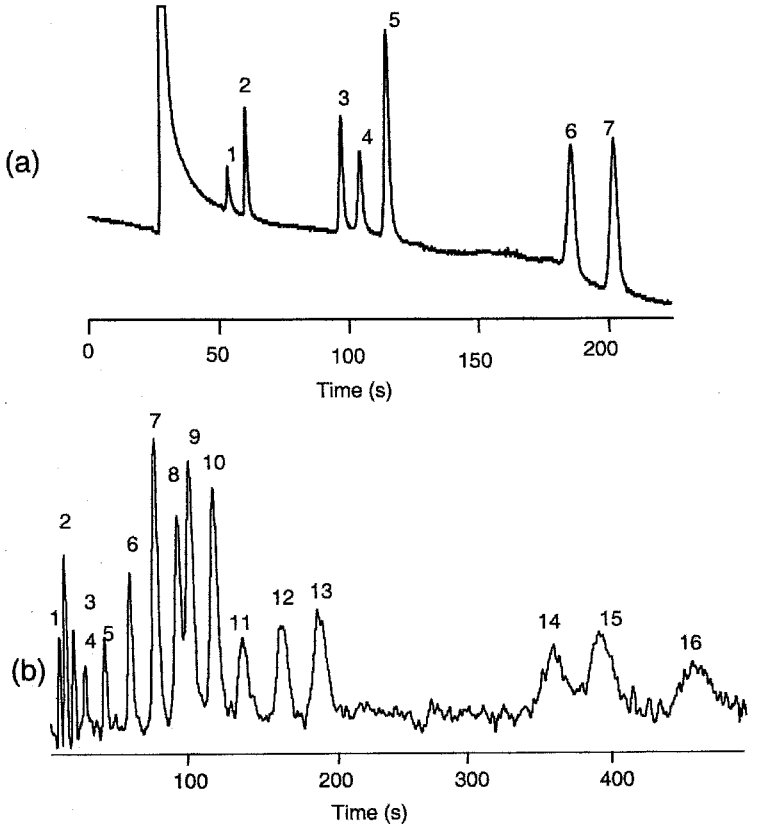

FIGURE 5.11 Chromatograms obtained with a SAW sensor (a) and a chemiresistor detector (b). Both detectors were microfabricated and have dead volumes of about 5 μl. The SAW sensor uses a 65-nm-thick film of polyisobutylene as the sensing element. The chemiresistor uses a spray-coated film of Au nanoparticles encapsulated with a self-assembled monolayer of n-C_8–S–Au. Atmospheric-pressure air was used as carrier gas for both chromatograms. Compounds for chromatogram (a) are 1, benzene; 2, trichloroethylene; 3, toluene; 4, n-octane; 5, tetrachloroethylene; 6, ethylbenzene; 7, p-xylene. Compounds for chromatogram (b) are 1, acetone; 2, 2-butanone; 3, benzene; 4, 2,5-dimethylfuran; 5, toluene; 6, tetrachloroethylene; 7, chlorobenzene; 8, ethylbenzene; 9, m-xylene; 10, styrene; 11, isobutylbenzene; 12, 1,2-dichlorobenzene; 13, heptaldehyde; 14, cumene; 15, α-pinene; 16, d-limonene.

Spectral acquisition rates of at least several hundred full-mass-range spectra per second can be achieved. High acquisition rates are needed in order to track the very narrow chromatographic peaks often associated with HSGC using short columns and high carrier-gas flowrates.

Spectral continuity refers to the fact that ion abundance ratios for the different masses in the spectrum are the same for all points on the chromatographic peak. Single-ion abundances vary as the vapor pressure in the ion source changes

during the passage of the component band through the ion source, but since ions of all mass-to-charge ratios are injected simultaneously into the mass analyzer (ion drift tube), the ion abundance ratio for any pair of mass-to-charge ratios is constant. Scanning mass analyzers including quadrupole and ion trap devices produce skewed spectra because the sample component vapor pressure in the ion source changes during the spectral scan. Thus, the mass spectra appear qualitatively different depending on whether the vapor pressure in the ion source is increasing or decreasing during the mass scan.

With constant ion abundance ratios for a peak containing only a single component, changes in the ratios across the peak profile indicate the presence of more than one component in the peak, and provide the basis for completely automated peak-finding and spectral deconvolution algorithms for severely overlapping peaks from unknown mixtures. This obviates the need for complete chromatographic separation and can thus dramatically decrease analysis time (32).

Figure 5.12 shows a portion of the chromatogram from a high-speed separation using TOFMS detection (Pegasus II, LECO Instruments, St. Joseph, MI). The

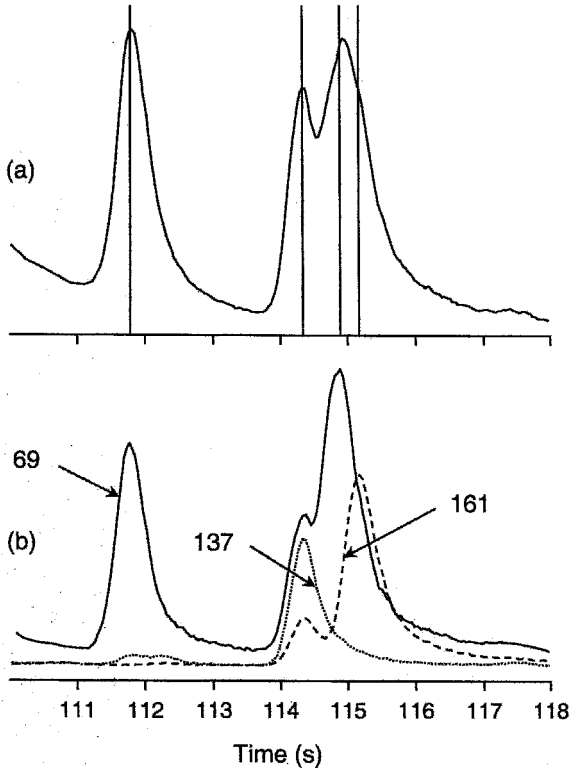

FIGURE 5.12 Chromatograms using TOFMS detection: (a) total-ion chromatogram and (b) extracted-ion chromatograms for three mass-to-charge ratios. Vertical lines indicate peak apex locations determined by automated peak-finding software.

spectral acquisition rate was 25 spectra per second. Chromatogram (a) shows the total-ion current and provides a single-channel measurement that is comparable to an FID chromatogram. Only three peaks are seen while four components are present. Very severe peak overlap occurs, and it would not be possible to obtain quantitative information about the three overlapping components without a much more complete separation. The vertical lines indicate the apices of the four peaks found using automated peak-finding software. Chromatogram (b) shows extracted ion chromatograms for three different mass-to-charge ratios (m/z) that are indicated by the numbers with arrows pointing to the three plots. These ions were chosen for display because 137 and 161 are relatively unique for individual components, and thus they clearly show how the shapes of the overlapping peaks can be defined by monitoring the unique-ion masses. A spectral deconvolution algorithm was used to obtain the pure mass spectra for the individual components from which component identification was obtained by a spectral match algorithm with a standard mass spectral library.

5.10 DATA SYSTEMS

Electrometer/amplifiers and data acquisition systems for many conventional GC instruments are too slow and contribute excessive extracolumn band broadening. Amplifiers often are highly damped to reduce measurement noise and thus reduce detection limits. However, this can result in severe peak tailing for narrow peaks. To minimize extracolumn band broadening and band distortion for HSGC, electrometer/amplifier systems should have time constants on the order of 10 ms, and data acquisition systems should operate with sampling rate on the order of 100 Hz. This requirement is easily achieved with modern electronic devices, and some commercially available GC instruments satisfy this requirement. For very fast separations (a few seconds or less), even smaller time constants and higher sampling rates may be required.

5.11 HIGH-SPEED TEMPERATURE PROGRAMMING

Until recently (as of 2003) most HSGC work has used isothermal conditions with convection oven instruments, and this has limited applications to mixtures spanning a relatively small boiling point range. The high-speed analysis of mixtures spanning a wide boiling point range can be accomplished only with high-speed temperature programming. Only recently has equipment become available commercially for high-speed temperature programming. Emerging technologies can obtain linear programming rates of at least 1000°C/min. With these conditions, a program ranging from 50 to 350°C (near the upper temperature limit for most bonded-phase fused-silica columns) is complete in 18 s.

5.11.1 Limitations of Convection Ovens

Convection ovens for conventional GC have changed relatively little in several decades. Better temperature controllers and higher-power heaters have resulted

in modest increases in maximum linear heating rates. However, over a wide temperature range (50–350°C), 50°C/min is about the highest ramp rate that is linear over the entire temperature range. Substantially higher linear ramp rates, up to about 100°C/min, can be obtained at lower temperatures.

Most conventional GC applications use temperature-programming rates that are smaller than need be, resulting in substantially longer analysis times with little increase in column resolving power relative to that obtained with faster temperature programming and shorter analysis times. Figure 5.13 shows chromatograms of a mixture of normal alkanes from *n*-C7 to *n*-C19 obtained with a programming rate of 50°C/min using a convection oven instrument. Chromatogram (a) was obtained with a 25-m-long, 0.25-mm-i.d. thin-film column operated in hydrogen with an average carrier-gas velocity of 100 cm/s. For chromatogram (b) the

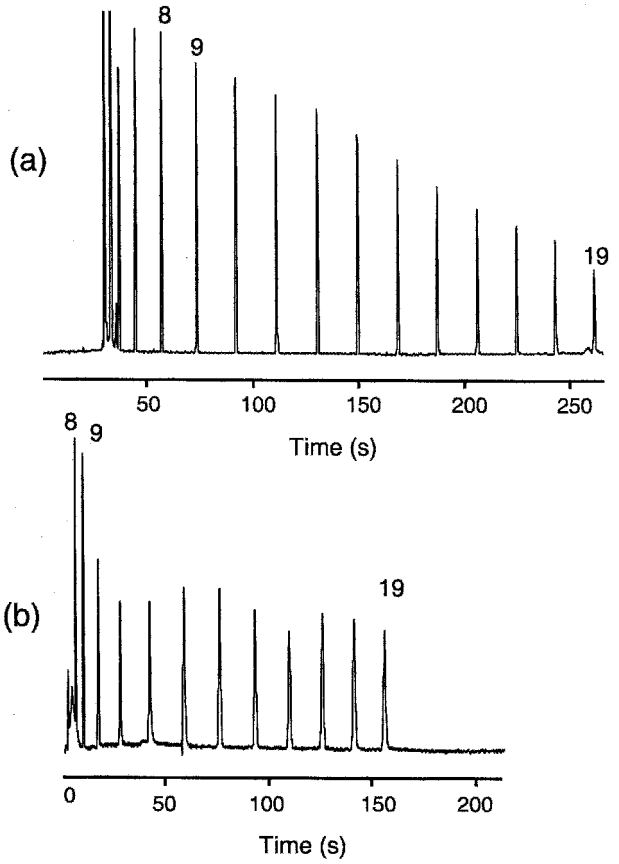

FIGURE 5.13 Temperature-programmed chromatograms of an *n*-alkane mixture (*n*-C7–*n*-C19) with a programming rate of 50°C/min: (a) 25-m-long, 0.25-mm-i.d. thin-film column with an average carrier-gas velocity of 100 cm/s; (b) 4.0-m-long, 0.25-mm-i.d. thin-film column with a gas velocity of 200 cm/s.

same column was used but the length was reduced to 4 m and the gas velocity increased to 200 cm/s.

For the 25-m column, the separation is complete in about 270 s. For the 4-m column at the higher gas velocity, the analysis time is reduced to 160 s, but the peak capacity and thus resolving power is substantially reduced. Note that the holdup times for chromatograms (a) and (b) are 25 and 2.0 s, respectively. For an isothermal separation, this would result in a 12.5-fold reduction in analysis time using the shorter column at the higher gas velocity. However, with the relatively high temperature-programming rate of 50°C/min the reduction in analysis time is less than twofold. Thus, the programming rate is more important in controlling retention times than the column length and flowrate.

Oven cooling is a major problem for HSGC with convection oven instruments. Convection ovens typically have large thermal mass, and cooling times are several minutes or more. This poses major limitations on instrument cycle time. Some instruments have provisions for more rapid cooling with cryogenic fluids. However, even in these cases, oven cooling may dominate instrument cycle time for HSGC.

5.11.2 At-Column Heating

High-speed column heating is obtained by applying heat at the column rather than suspending the column in a large convection oven (8,18). This (at-column heating) is accomplished by the use of a heater wire collinear with or wrapped around the fused-silica capillary column or by the use of a cylindrical metal heater sheath surrounding the column. An electrical current through the wire or sheath is used to heat the column. Usually a sensor wire collinear with the column is used to monitor the average column temperature and as part of the feedback loop used to control the column temperature. Early attempts at at-column heating of fused-silica columns used columns painted with an electrically conductive gold paint or other metal film. However, good column-to-column reproducibility and robustness were difficult to achieve.

Figure 5.14 shows the high-speed separation of a normal alkane mixture (*n*-C10–*n*-C20 plus *n*-C22, *n*-C24, *n*-C26, and *n*-C28) using temperature-programming rates of 100°C/min (a) and 600°C/min (b). A 6-m-long, 0.25-mm-i.d. thin-film column was used with hydrogen carrier gas at an average velocity of 100 cm/s. Thus the holdup time was 6 s. The analysis is complete in only 37 s with the 600°C/min programming rate.

5.11.3 Effects of Heating Rate on Analysis Time and Peak Capacity

Since retention factors change continuously during a temperature-programmed run, and with sufficiently high programming rate all peaks have nearly the same widths, measures of column efficiency and resolving power described for isothermal separations cannot be applied directly to the case of temperature-programmed GC. However, the relatively constant peak widths allows for the use of a simple

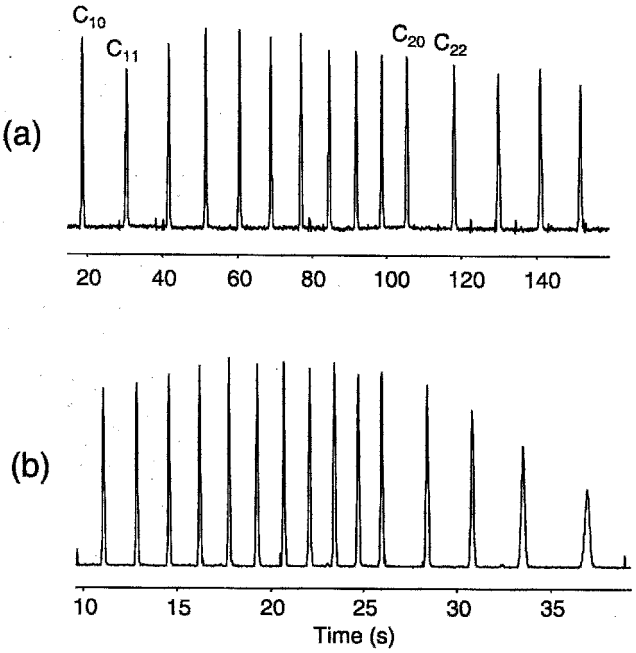

FIGURE 5.14 Temperature-programmed chromatograms of an *n*-alkane mixture (*n*-C10–*n*-C20 plus *n*-C22, *n*-C24, *n*-C26, and *n*-C28) with programming rates of 100°C/min (a) and 600°C/min (b) with a 6.0-m-long, 0.25-mm-i.d. thin-film column and an average carrier-gas velocity of 100 cm/s.

empirical method for the reliable calculation of peak capacity. The separation number SN (also called Trennzahl number TZ) (33) describes the number of peaks that will fit between the peaks of two reference compounds with a resolution of 1.18 if the reference peaks are separated by Δt_R and their widths at half-height are $(w_h)_1$ and $(w_h)_2$:

$$\mathrm{SN} = \left(\frac{\Delta t_R}{(w_h)_1 + (w_h)_2} \right) - 1 \tag{5.12}$$

Usually, adjacent *n*-alkanes are used for the reference compounds so that the separation number is defined locally over a relatively small region of the chromatogram extending from *n*-*C*(*i*) to *n*-*C*(*i* + 1). Values of peak capacity n_{pR} for any specified resolution R_s can be found from the following equation:

$$n_{pR} = \frac{1.18}{R_s} \mathrm{SN} \tag{5.13}$$

If SN values are summed over the range of *n*-alkanes *n*-C(*i*)–*n*-C(*j*) that span the retention time range for a mixture of target compounds and the peaks for the

reference compounds added, the total peak capacity SN_T for the separation can be found:

$$SN_T = \sum_{i}^{j}(SN + 1) \tag{5.14}$$

Values of SN decrease with decreasing column length (fewer theoretical plates) and with increasing boiling points of the reference compounds (34). Figure 5.15 shows plots of cumulative SN (peak capacity) from Equation 5.14 versus retention time for a mixture containing *n*-C8–*n*-C19 with the 25-m-long, 0.25-mm-i.d column used for Figure 5.13a with temperature-programming rates of 20, 30, 40, and 50°C/min. These plots show how peak capacity measured as SN values accumulates with time during a separation.

For example, for the 20°C/min. programming rate, *n*-C19 elutes in about 490 s, and the total peak capacity in the chromatogram is about 290 peaks. For the 50°C/min programming rate, the analysis time is reduced to 260 s, and the peak capacity is reduced to about 240 peaks. The slopes of these plots give the rate of peak capacity generation at any point in the corresponding chromatograms. The tradeoffs between analysis time and peak capacity are clearly seen in Figure 5.15.

Figure 5.16 shows similar plots of cumulative peak capacity versus time for the mixture used in Figure 5.14 with at-column heating of a 6-m-long column and programming rates of 60, 200, 400, and 600°C/min. With a 60°C/min programming rate, *n*-C28 elutes in about 240 s with a total SN from *n*-C10 to *n*-C28 of about 150 peaks. With a 600°C/min programming rate, *n*-C28 elutes in less than 40 s, but the total SN has been reduced to less than 80 peaks.

High-speed separations of wide-boiling-point-range mixtures require fast temperature programming. However, if the programming rate is too high, a serious

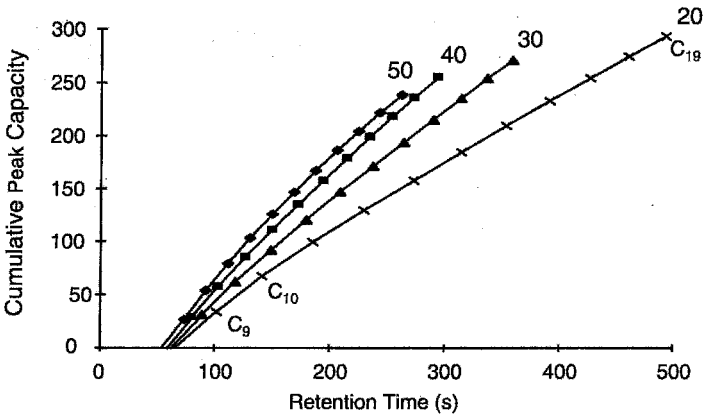

FIGURE 5.15 Plots of cumulative peak capacity (SN_T) versus retention time for an *n*-alkane mixture (*n*-C8–*n*-C19) for temperature-programming rates of 20, 30, 40, and 50°C/min using a 25-m-long, 0.25-mm-i.d. column with an average carrier-gas velocity of 100 cm/s.

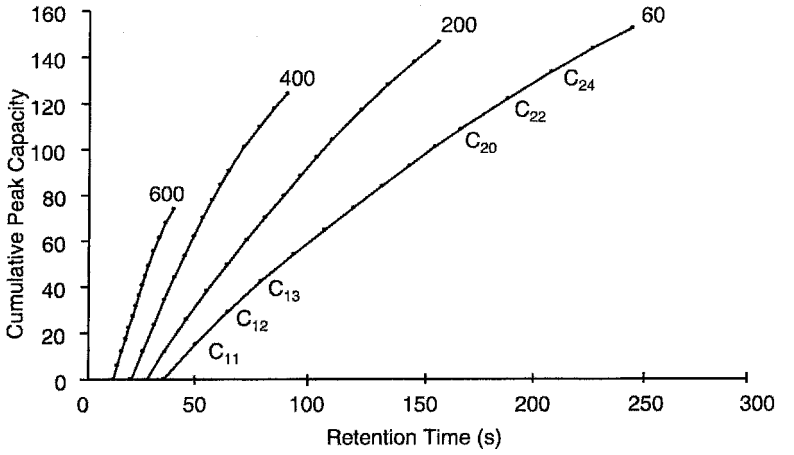

FIGURE 5.16 Plots of cumulative peak capacity (SN_T) versus retention time for an *n*-alkane mixture (*n*-C10–*n*-C20 plus *n*-C22, *n*-C24, *n*-C26, and *n*-C28) for temperature-programming rates of 60, 200, 400, and 600°C/min using a 6.0-m-long, 0.25-mm-i.d. column with an average carrier-gas velocity of 100 cm/s.

loss in peak capacity will occur as seen in Figures 5.15 and 5.16. The explanation for the loss in peak capacity is that retention factors may decrease so much before elution that the downstream end of the column becomes very inefficient (too little interaction with the stationary phase). The key factor is the column temperature increase during a time interval equal to the holdup time. Large losses in peak capacity can be avoided if the temperature increase is no more than about 10–20°C per holdup time interval (18).

Using a upper limit of 15°C per holdup time interval and a 10-m-long column operated with an average carrier gas velocity of 100 cm/s, the holdup time is 10 s, and the maximum temperature programming rate should not exceed about 90°C/min. Note that this value exceeds the capabilities of conventional GC instruments using convection ovens. Higher average carrier-gas velocities allow for the use of higher temperature-programming rates, but if the velocity is too high, reduced column efficiency will again result in reduced peak capacity. The best temperature-programming rate for a high-speed separation depends on the application. For relatively simple mixtures where peak capacity is not an issue, higher programming rates can be used with corresponding reductions in analysis time.

PART 4 SELECTIVITY ENHANCEMENT METHODS

While high-speed GC with open tubular columns was demonstrated for small sets of compounds as early as 1962 (2), only recently (at the time of writing) has this technology been successfully applied to more specific applications involving

more than a few target compounds. The most significant obstacle to the high-speed gas chromatographic separation of more complex mixtures is the high probability of peak overlap caused by the reduced peak capacity associated with the conditions (short, open tubular columns and high carrier-gas flowrates) needed for fast separations.

5.12 COPING WITH REDUCED PEAK CAPACITY

While open tubular columns are comparable with packed columns in terms of efficiency under optimal flowrate conditions, longer open tubular columns provide more theoretical plates and thus have greater resolving power. A typical 30-min-long packed-column separation with a 2-m column will generate a typical peak capacity of 30–100 peaks. A 50-m-long open tubular column can generate a peak capacity of several hundred peaks in the same timeframe.

Prior to the widespread use of open-tubular columns, hundreds of different stationary phases were in use for packed-column GC. This was necessary because of the relatively poor resolving power of packed columns. Usually, complex chemical cleanup procedures, such as solvent extraction, were needed to reduce the number of potential chromatographic interferences. Stationary phases often were developed for very specific groups of target compounds. Once the use of open tubular columns became widespread, much greater resolving power became available; chromatographic interferences became less common, and the need for a very large number of stationary phases was reduced. More universal columns with high resolving power were marketed with lengths in the range between 15 and 100 m. The need for extensive sample cleanup was reduced, and the number of commercially available stationary phases fell dramatically.

Method development for GC with open tubular columns often is reduced to the relatively simple task of choosing one of a few stationary phases, and using an isothermal temperature or temperature-programming regime with a column of sufficient length to just resolve the most difficult component pair (critical pair). Usually, many other adjacent component pairs are very overseparated, with the result that chromatograms often contain extensive empty space. When relatively short lengths of open tubular column are used at higher-than-usual carrier-gas flow rates for HSGC, resolving power is reduced, and peak capacities are again comparable to values achieved with packed columns, but with analysis times that typically are an order of magnitude or more less than with packed column.

5.13 ADJUSTMENT OF SELECTIVITY

The key to applying HSGC to more complex mixtures is to achieve enhanced selectivity. By the adjustment of selectivity, more control over the structure of chromatograms can be achieved, and it is possible to utilize the available peak capacity with greater efficiency. If the required peak capacity can be reduced by

a factor of 2, the required column length can be reduced by a factor of 4 (see Equation 5.3), and by Equation 5.1, analysis time can be reduced by a factor of 4.

5.13.1 Mixed Stationary Phases

To extend the usefulness of packed columns, stationary phase materials have been mixed in varying proportions, and the proportion adjusted to enhance the selectivity for a specified set of target compounds (35). The pattern of peak retention times produced by the column can be adjusted within the range of patterns produced by the individual stationary-phase materials. The effects of each phase in the mixture on the retention of the resulting peaks are additive if retention for each mixture component is expressed as its retention factor for the individual stationary phases and if the total volume of stationary phase is constant.

For a mixture of two stationary phases, we obtain

$$k_0 = \phi_A k_A + \phi_B k_B \tag{5.15}$$

$$\phi_A + \phi_B = 1.0 \tag{5.16}$$

where k_0 is the component retention factor for the mixed-phase column; k_A and k_B are the retention factors for columns using stationary-phase materials A and B, respectively; and ϕ_A and ϕ_B are the volume fractions of the stationary-phase materials in the mixed stationary phase. After coating the stationary-phase support particles with the mixed stationary phase, the column is packed.

An alternative and more convenient method uses a mixture of support particles coated with the different stationary-phase materials. For a specified solute, the same k_0 value is obtained as when the phases are mixed prior to coating the stationary-phase support particles if the volume of each stationary-phase material is the same. Equations 5.15 and 5.16 can be combined to obtain Equation 5.17:

$$k_0 = k_B + \phi_A(k_A - k_B) \tag{5.17}$$

Thus, for each compound in the mixture, a plot of k_0 versus the volume fraction ϕ_A should give a straight line with slope equal to $k_A - k_B$ and intercept equal to k_B. An alternative expression is found in Equation 5.18:

$$k_0 = k_A + \phi_B(k_B - k_A) \tag{5.18}$$

Selectivity optimization procedures for mixed stationary phases involve the selection of the values of ϕ_A and ϕ_B that will give the highest resolution of the component pair that is most difficult to separate (the critical pair). In Figure 5.17a, values of k_0 are plotted against ϕ_A for a mixture of five arbitrary compounds labeled 1–5. The left vertical axis ($\phi_A = 0$) corresponds to the retention factor values for a column using only stationary phase B, and the right vertical axis corresponds to using only stationary phase A. Note that the elution order on phases A and B are 3,1,2,4,5 and 1,2,5,4,3, respectively. These limiting points can

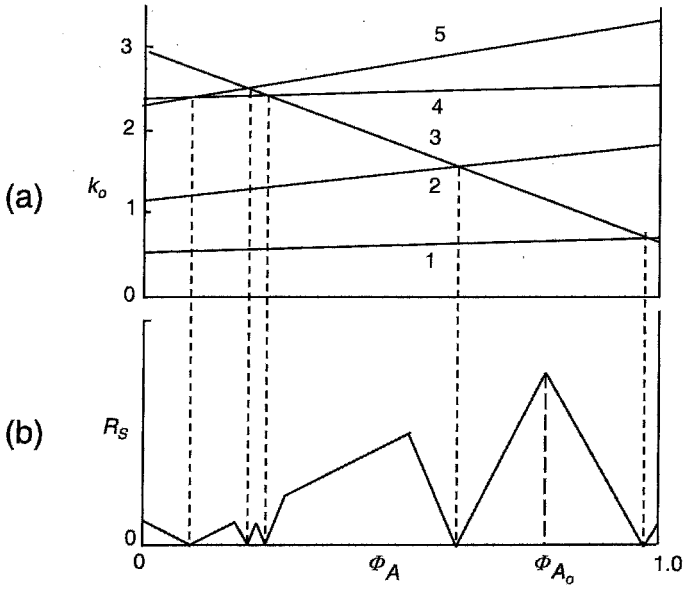

FIGURE 5.17 Plots of k_0 versus Φ_A for an arbitrary five-component mixture (a) and the resulting resolution window diagram (b). Vertical broken lines indicate Φ_A values that result in coelution of the corresponding component pairs. The value of Φ_{A0} gives the volume fraction of stationary phase A that will give the greatest resolution of the critical pair.

then be connected with straight lines using Equation 5.17. Plots are observed with positive and with negative slopes. A positive slope indicates that the component retention factor is larger with stationary phase A, and a negative slope indicates that the component retention factor is larger with stationary phase B. Note that components 1 and 3 may be difficult to resolve with phase A, and components 4 and 5 may be difficult to resolve with phase B. Thus, neither stationary phase alone may be adequate for a complete separation.

The vertical broken lines in Figure 5.17a indicate the ϕ_A values for which pairs of plots cross. Since the k_0 values for the corresponding pair of components are the same at these crossing points, a mixed-phase column using one of these ϕ_A values would result in the coelution of the corresponding pair of compounds. Between the vertical lines are values of ϕ_A that will not result in complete coelutions, but may or may not provide adequate resolution of all component pairs.

Optimization procedures are used to determine which of these ϕ_A values will obtain the greatest resolution of the critical pair (36). Figure 5.17b shows a resolution window diagram where the resolution R_s of the critical pair is plotted against the volume fraction of phase A. The zero-resolution points are defined by the ϕ_A values that result in the crossing of the various plot pairs in Figure 5.17a. Between the zero-resolution points are windows of various amplitude, which

correspond to the resolution of the critical pair. The critical pair changes at the apex of each window. The ϕ_A value giving the largest window apex (ϕ_{A0}) gives the greatest resolution of the critical pair and thus is the best choice for ϕ_A.

5.13.2 Designer Stationary Phases

New stationary phases are now being designed for HSGC with the aid of computer models. Dorman, et al. (37) report on the use of empirical modeling methods for the development of new stationary phases for the fast separation of certain sets of target compounds including pesticides, explosives, PCBs, and dioxins. Computer models are used to simulate stationary phase selectivity for phases containing multiple functionalities. The models use retention data for the target compounds with columns having different functionalities. A retention surface is generated for each compound, and the intersection of these surfaces define the coelutions. Using a column designed with these models, 18 explosive compounds were completely separated in less than 8 min.

5.13.3 Tunable/Programmable Selectivity with Tandem Capillary Columns

Two capillary columns with different stationary phases can be combined in series (tandem) to obtain tunable selectivity. The length ratio of the columns can be changed to change the contributions that the two columns make to the overall selectivity (k_0 values) in the same way that the volume ratios of the stationary phases can be changed with mixed-phase packed columns (38). The left portion of Figure 5.18 shows a tandem column ensemble consisting of columns C_A and C_B. The right portion of the figure shows solute band position versus time plots for another arbitrary mixture of five components labeled 1–5. For these plots, injection into C_A occurs at the lower left corner (zero time), and elution from the column ensemble occurs along the top horizontal line. The broken horizontal line at the center of the plots corresponds to the column junction point. The band trajectory plots in this simplified illustration are shown as straight lines. In fact, some curvature occurs due to carrier-gas acceleration along the column.

As each mixture component migrates across the column junction point, the migration velocity (slope) of each component changes abruptly due to the different retention factors for the two columns. Note that for column C_A, components 2 and 3 have nearly the same slope, and for C_B, 2 and 4 have nearly the same slope. Thus, peaks for 2 and 3 would coelute using only column C_A, and 2 and 4 would coelute using only column C_B. The tandem combination of the two columns provides a unique selectivity, which results in the complete separation of the mixture.

A much more convenient way to adjust the selectivity of a series-coupled (tandem) column combination is to provide adjustable carrier gas pressure at the column junction point (14,15,39–41). This is illustrated in Figure 5.19. An electronic pressure controller PC is used to adjust the junction point pressure.

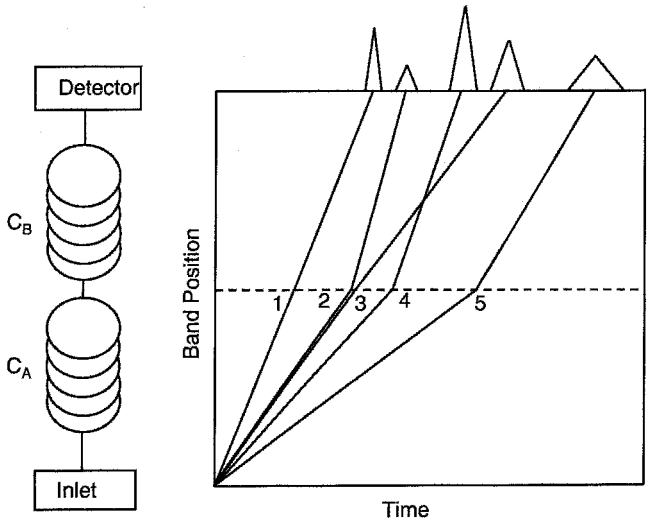

FIGURE 5.18 Series-coupled column ensemble for tunable selectivity. Selectivity is adjusted by adjusting the length ratio of the two columns. The plots of solute band position versus time for compounds 1–5 show how the column combination obtains a unique selectivity.

Detector D_1 monitors the output from the tandem column ensemble. Detector D_2 monitors a small fraction of the effluent from C_A. Capillary restrictor R controls the amount of the C_A effluent directed to D_2. This second detector is optional, but it is useful for method development.

Changing the carrier-gas pressure at the column junction point changes the residence times of all components in the two columns. For example, if the junction point pressure is increased, the pressure drop along C_A decreases, and the drop along C_B increases. This results in decreased carrier gas flow and thus longer solute residence times in C_A. Carrier-gas flow increases in C_B, resulting in shorter residence times. This increases the contribution that C_A makes to the overall selectivity of the column combination. Optimization is based on the use of resolution window diagrams as shown in Figure 5.17 except the stationary-phase volume fractions ϕ_A and ϕ_B are replaced with the holdup time fractions f_A and f_B for the two columns as given in Equations 5.19 and 5.20:

$$f_A = \frac{t_{MA}}{t_{MA} + t_{MB}} \tag{5.19}$$

$$f_B = \frac{t_{MB}}{t_{MA} + t_{MB}} \tag{5.20}$$

where t_{MA} and t_{MB} are the holdup times for the respective columns in the ensemble.

Figure 5.20 shows a portion of high-speed chromatograms containing peaks from six components. Note that the six components elute in a time window of

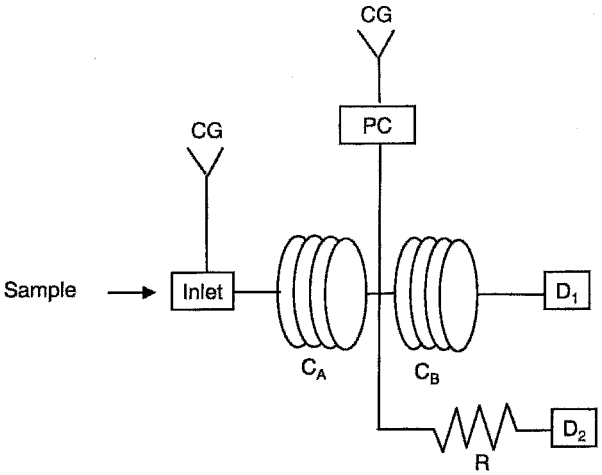

FIGURE 5.19 Pressure-tunable column ensemble. Columns C_A and C_B use different stationary phases. Electronic pressure controller PC is used to adjust the carrier gas pressure at the column junction point. Detector D_1 monitors the final chromatogram from the column ensemble, and D_2 monitors a small fraction of the effluent from C_A. Carrier gas is provided at points CG.

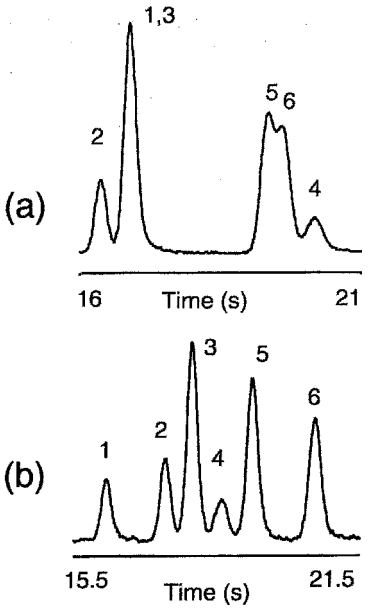

FIGURE 5.20 Portions of chromatograms showing the effects of a change in the junction point pressure using the apparatus of Figure 5.19. The column ensemble consists of a 5.0-m-long, 0.25-mm-i.d. dimethylpolysiloxane column followed by a 5.0-m-long, 0.25-mm-i.d. poly(ethylene glycol) column.

about 6 s. The only difference between the two chromatograms is the carrier-gas pressure at the column junction point. Note that the pattern of peaks is very different for the two junction point pressures, and for chromatogram (b), a complete separation is achieved.

For a mixture containing n components, the number m of unique peak pairs is given by

$$m = \frac{n^2 - n}{2} \tag{5.21}$$

For the five-component mixture described in Figure 5.17, there are 10 unique peak pairs. For a 30-component mixture, there are 435 unique peak pairs. As n increases, it becomes less likely that any combination of the two stationary phases (junction point pressure) will result in a complete separation.

Improved separation quality for more complex mixtures often can be obtained with programmable selectivity where the junction point pressure of the tandem column ensemble is changed one or more times on the fly during the separation (42). Initially, the junction point pressure is set to give optimal separation conditions for the first group of peaks to elute from the column ensemble. After these component bands have migrated into the second column, the junction point pressure can be changed to a value more appropriate for the next group of components, which are still in the first column. This process can be repeated as many times as necessary.

An example is shown in Figures 5.21 and 5.22 where a 20-component mixture is separated isothermally in about one minute using a 12-m-long, 0.25-mm-i.d. thin film column ensemble consisting of 6.0 m of a nonpolar dimethyl polysiloxane column C_A followed by 6.0 m of a polar polyethylene glycol column C_B. The plots of solute band position versus time were obtained by spread sheet calculations, which use as input retention factors for all components on the individual columns as well as the column dimensions, the inlet, outlet (1.0 atm) and junction-point pressures and the carrier-gas (hydrogen) viscosity at the column temperature. The slight curvature in the plots is the result of carrier-gas acceleration from inlet to outlet.

No single junction point pressure was found adequate for a complete separation of the mixture. However, using window diagram analysis, a pressure was found that gave a complete separation of the first 14 compounds. The resulting separation is shown in Figure 5.21. Note the coelution of peak pairs 17,19 and 16,20. For Figure 5.22, the same initial junction-point pressure was used as for Figure 5.21, but the pressure was reduced 21 s after injection. At this time, the first 14 compounds have either eluted from the column ensemble or have migrated into the second column, but components 15–20 are still in the first column. Note that the slopes of the band position/vs time plots change significantly after the junction point pressure change, and this results in a large change in the final elution pattern for peaks 15–20. In Figure 5.22, all 20 components are completely separated. The inset shows the first nine components and an unretained peak (U) on an expanded timescale.

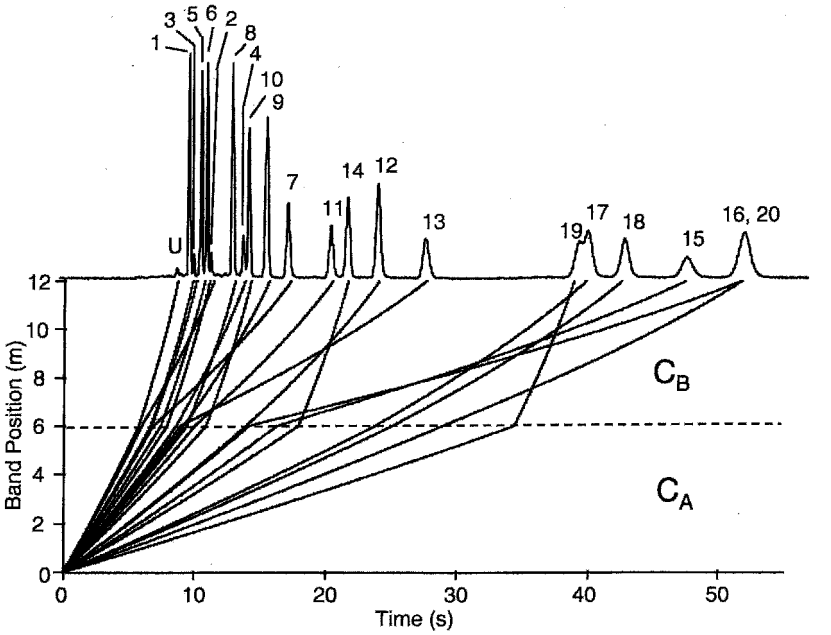

FIGURE 5.21 High-speed isothermal separation of a 20-component mixture using a pressure-tunable column ensemble. The plots of band position versus time were obtained from the retention factors for the mixture components on the two separate columns along with the column dimensions and the inlet, outlet and junction point pressures. A window diagram was used to determine the junction point pressure for the complete separation of the first 14 components. Compounds are 1, *n*-pentane; 2, methyl alcohol; 3, 2,2-dimethylbutane; 4, 1,1,1-trichloroethane; 5, cyclopentane; 6, *n*-hexane; 7, *n*-propyl alcohol; 8, cyclohexane; 9, benzene; 10, *n*-heptane; 11, 1,2-dichloropropane; 12, toluene; 13, *n*-butyl alcohol; 14, *n*-octane; 15, 2-hexyl alcohol; 16, *n*-pentyl alcohol; 17, ethylbenzene; 18, *m*-xylene; 19, *n*-nonane; 20, *o*-xylene.

5.13.4 Pulse Flow Modulation with Tandem Capillary Columns

The limitation of tunable and programmable selectivity with tandem capillary columns using electronic pressure control at the column junction point is that changing the junction point pressure results in a change in the ensemble elution pattern for the entire mixture or a subgroup of the mixture, and under optimal conditions, any pressure change used to enhance the resolution of one component pair will degrade the resolution of another component pair.

A solution to this problem is to replace the electronic pressure controller with a low-dead-volume computer-controlled valve and a source of carrier gas at some preset pressure. Normally the valve is closed, and the column junction point pressure is the natural pressure that occurs at the column junction point in the absence of any additional connections. When the valve is opened, the junction point pressure assumes the preset value of the additional carrier-gas

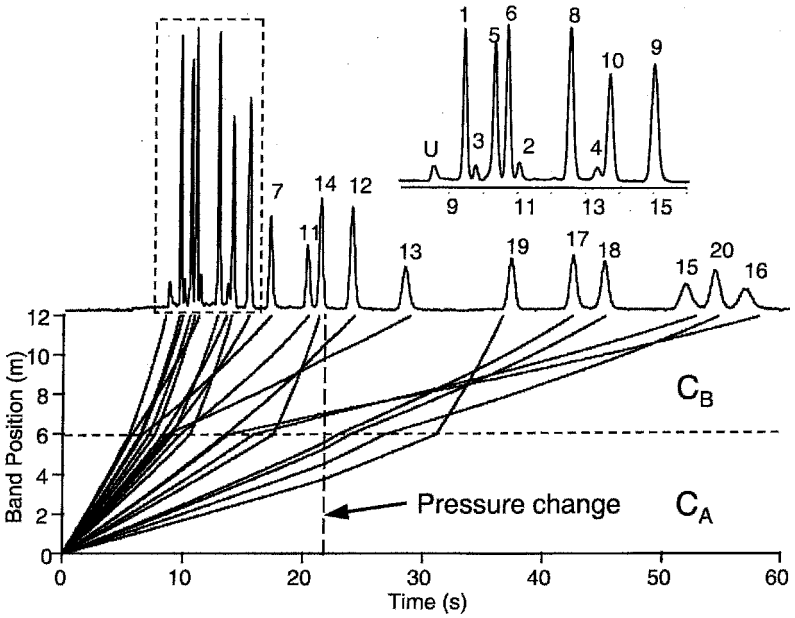

FIGURE 5.22 High-speed isothermal separation of the 20-component mixture from Figure 21 except that the junction-point pressure was decreased 21 s after injection to obtain the complete separation of the mixture.

source. Usually, the valve is open for only a few seconds in order to enhance the resolution of a particular component pair. Thus, the carrier gas in the two columns undergoes a pulsed-flow modulation (43,44).

A particularly attractive version of pulsed-flow modulation uses the GC inlet pressure as the preset pressure (45,46). Thus, when the valve is opened, both ends of column C_A are at the same pressure, and carrier-gas flow in C_A stops (stop-flow operation). Stop-flow operation is used to enhance the resolution of a targeted component pair without significantly changing the elution pattern and resolution of other components in the mixture. The concept is illustrated by the band trajectory plots shown in Figure 5.23 for a pair of components labeled 1 and 2 that are completely separated by the first column but coelute from the column ensemble. The solid-line plots are for the case without a stop-flow pulse, and the broken-line plots for the case with a 5-s-wide pulse occurring at the time indicated by the vertical lines.

For case (a), the pressure pulse is applied with both components are in C_A. Both bands stop during the pulse, and the peaks in the ensemble chromatogram are shifted to a later time but without significant change in resolution. For case (b), the pulse is applied after the first of the component bands has migrated across the column junction point and is in C_B, but the band for the other component is still in the first column. The band in C_A stops for the duration of the pulse, while the band in C_B migrates more rapidly during the pulse. The result is

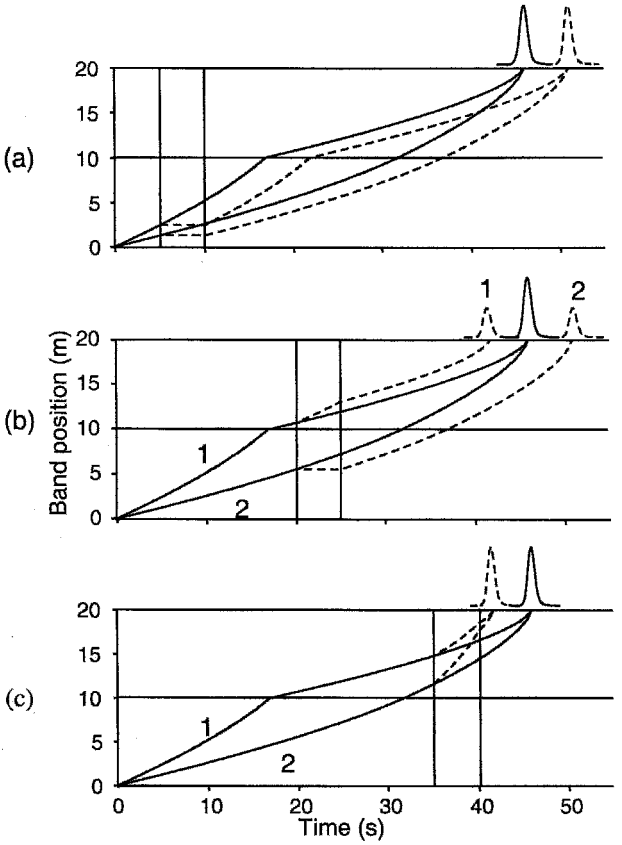

FIGURE 5.23 Plots of band position versus time for a two-component mixture (1 and 2) illustrating stop-flow operation. Solid-line plots are for the case without a stop-flow pulse, and broken-line plots are for the case with a 5-s-wide stop-flow pulse indicated by the vertical lines. (a) Stop-flow pulse applied when both components are in C_A; (b) stop-flow pulse applied when component 1 is in C_B and component 2 is in C_A; (c) stop-flow pulse applied when both components are in C_B.

the complete separation of the components in the ensemble chromatogram. For case (c), the pulse is applied after both components have crossed the junction and are in C_B, and both peaks are shifted to shorter retention times, but with no significant change in resolution.

Figure 5.24 shows the high-speed separation of a 20-component pesticide mixture (plus one impurity peak). The 14-m-long, 0.18-mm-i.d. thin film column ensemble consists of 7.0 m of a trifluoropropylmethyl polysiloxane column followed 7.0 m of 5% phenyl dimethyl polysiloxane column segment. For chromatogram (a), no stop-flow pulses were used, and component pairs 2,3 and 10,11 coelute. For chromatogram (b), a single 2-s wide stop-flow pulse was used to enhance the resolution of peak pair 2,3. Note that the peak pattern and resolution

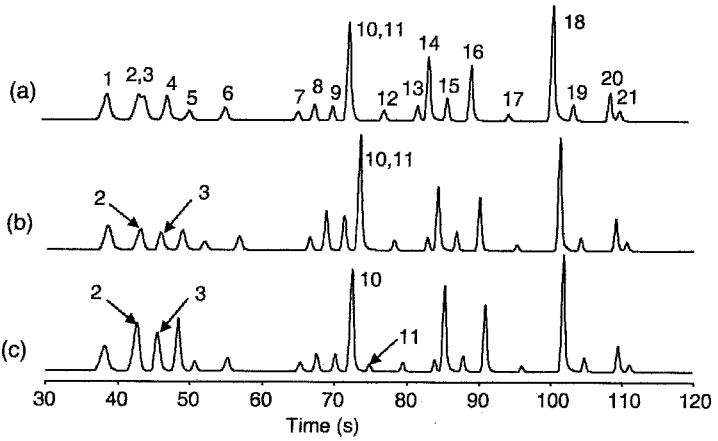

FIGURE 5.24 High-speed temperature-programmed separation of a 20-component pesticide mixture without stop-flow operation (a), with a 2-s-wide stop-flow pulse to separate components 2 and 3 and (c), and with two stop-flow pulses to separate component pairs 2,3 and 10,11. Components are 1, α-BHC; 2, β-BHC; 3, γ-BHC, 4, δ-BHC; 5, heptachlor; 6, aldrin; 7, heptachlor epoxide; 8, α-chlordane; 9, γ-chlordane; 10, 4,4′-DDE; 12, dieldrin; 13, endrin; 14, 4,4′-DDD; 15, endosulfan II, 16, 4,4′-DDT; 17, endrin aldehyde; 18, metoxychlor; 19, endosulfan sulfate; 20, impurity; 21 endrin ketone.

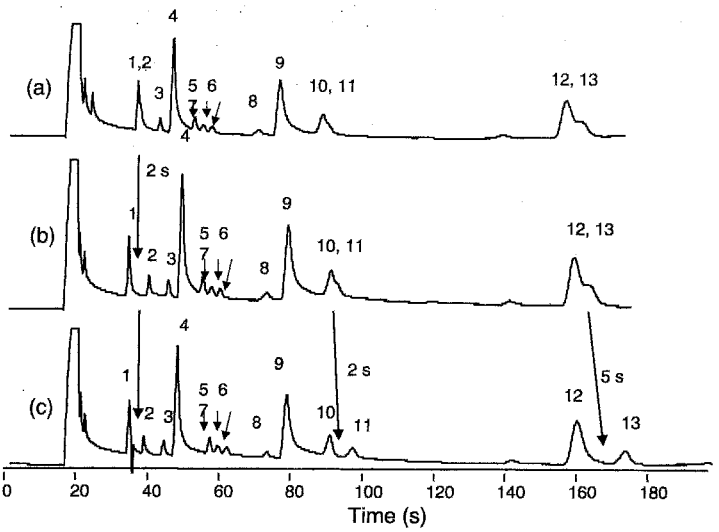

FIGURE 5.25 High-speed isothermal separation of a 13-component mixture without stop-flow operation (a), with a 2-s-wide stop-flow pulse to separate components (1 and 2), (b) and (c) with three stop-flow pulses to separate component pairs (1,2), (10,11), and (12,13). Vacuum outlet GC was used with an outlet pressure of 0.5 atm., and atmospheric-pressure air was used as carrier gas. Components are 1, ethyl acetate; 2, 2-butanone; 3, benzene; 4, 1-butanol; 5, trichloroethylene; 6, *n*-heptane; 7, 2,5-dimethylfuran; 8, 2,4-dimethylhexane; 9, 3-methyl-1-butanol; 10, toluene; 11, 2-methylheptane; 12, butylacetate; 13, chlorobenzene.

of the other components shows no significant change. For chromatogram (c), a second stop-flow pulse was added to enhance the resolution of component pair 10,11. With the two stop-flow pulses, a complete separation is achieved in about 110 s.

Figure 5.25 shows chromatograms of a 13-component mixture using a tandem column ensemble without stop-flow operation (a), with a single, 2-s-wide stop-flow pulse timed to enhance the resolution of component-pair 1,2 (b) and with a sequence of three stop-flow pulses to enhance the resolution of three component pairs (c) that all coelute from the column ensemble without stop-flow operation. These chromatograms were obtained with vacuum outlet GC using atmospheric-pressure air as the carrier gas (46). The 9.0-m-long, 0.25-mm-i.d. column ensemble consists of 4.5 m of 0.5-μm-thick dimethyl polysiloxane column followed by 4.5 m of 0.25-μm film trifluoropropylmethyl polysiloxane column. Since atmospheric-pressure air is used as the carrier gas, stop-flow operation can be obtained simply by opening the stop-flow valve to ambient air. Note that the sequence of stop-flow pulses greatly improves the separation quality while adding only about 10 s to the total analysis time.

PART 5 PORTABLE AND MINIATURIZED HSGC SYSTEMS

On-site analysis is becoming increasingly important, especially in the area of environmental monitoring. On-site monitoring reduces the risk of contamination, sample loss, and sample decomposition during transport. On-site monitoring also results in much shorter analysis turn-around times and thus allows for faster response to the analytical results. To be effective, an on-site instrument should be small, lightweight, and low-maintenance. In order to achieve these features, resolution and sensitivity often are compromised. New instrument designs and component manufacturing methods are coming on line that will result in the development of a new generation of high-performance portable and miniaturized instruments for HSGC. The use of microelectromechanical systems (MEMS) technologies for the manufacturing of microfabricated gas chromatographic components will result in very small, autonomous, low-cost instruments for environmental monitoring. The development of MEMS gas chromatographic components and systems for HSGC is in progress at several National laboratories and universities.

5.14 REQUIREMENTS FOR MINIATURIZED, AUTONOMOUS HSGC SYSTEMS

Completely autonomous GC instruments require no daily maintenance and can be placed in remote locations for long-term service. This requires battery operation, wireless communications, and freedom from tanks of compressed gases. To this

end, work is in progress to develop a high-performance micro-GC (μGC) that will have a volume of about 5 cm^3 and will operate with an average power consumption of less than 10 mW (47). To achieve complete autonomy, vacuum outlet GC will be used with ambient air as carrier gas. In addition, remote battery charging with radiofrequency transmission will be required.

The use of ambient air as a carrier gas poses several challenges. First, some stationary phases rapidly decompose in air. Poly(ethylene glycol) (wax) is a good example. Second, the high viscosity and relatively small binary diffusion coefficients for organic compounds in air result in low optimal gas velocity and rapid loss in efficiency for gas velocities much greater than the optimum value. This results in longer analysis times than can be achieved with hydrogen or helium. In addition, particulate material and water vapor may need to be removed.

Sensor array detection also is needed because these devices can be microfabricated with very low dead volumes; they require no support gases for their operation, and they can be fabricated with a variety of selectivities, which can be used for vapor recognition and for the deconvolution of overlapping peaks. This can reduce the resolution requirements for the column. Sensor detectors usually have lower sensitivity than do detectors incorporated in laboratory gas chromatographic instruments. Low detector sensitivity, coupled with the very low concentrations often associated with air monitoring, requires the use of a sorption preconcentrator for sample enrichment prior to separation and detection.

5.15 MICROELECTROMECHANICAL COMPONENTS FOR HSGC

5.15.1 Microfabricated Columns

Several processes have been described for the microfabrication of gas chromatographic columns using substrates of silicon, various metals, and even plastic materials (47–49). Etched silicon channels are most common. Reactive-ion etching is used to produce rectangular channels of precisely controlled dimensions. With these processes, narrow, deep rectangular cross-sectional channels can be obtained. For channels of high aspect ratios (ratio of channel depth to width) high column efficiency can be combined with relatively high volumetric flowrate (to reduce band broadening from dead volumes) and high surface area for increased stationary-phase volume.

Figures 5.26 and 5.27 show gas chromatographic columns made from etched silicon wafers and chromatograms for a 0.9-m-long column and a 3.0-m-long column, respectively. The channels are 150 μm wide and 240 μm deep. After etching, a glass coverplate is bonded to the silicon surface, and the channel is coated with stationary phase. Photomicrographs were made from silicon wafers that were sliced after etching. The channels have very vertical walls and nearly flat bottoms. For the 0.9-m-long columns, both spiral and serpentine channels were etched. The substrate size is 1.7 cm on a side. A penny is shown in the photographs as a size comparison. The 3-m-long column is etched as a double

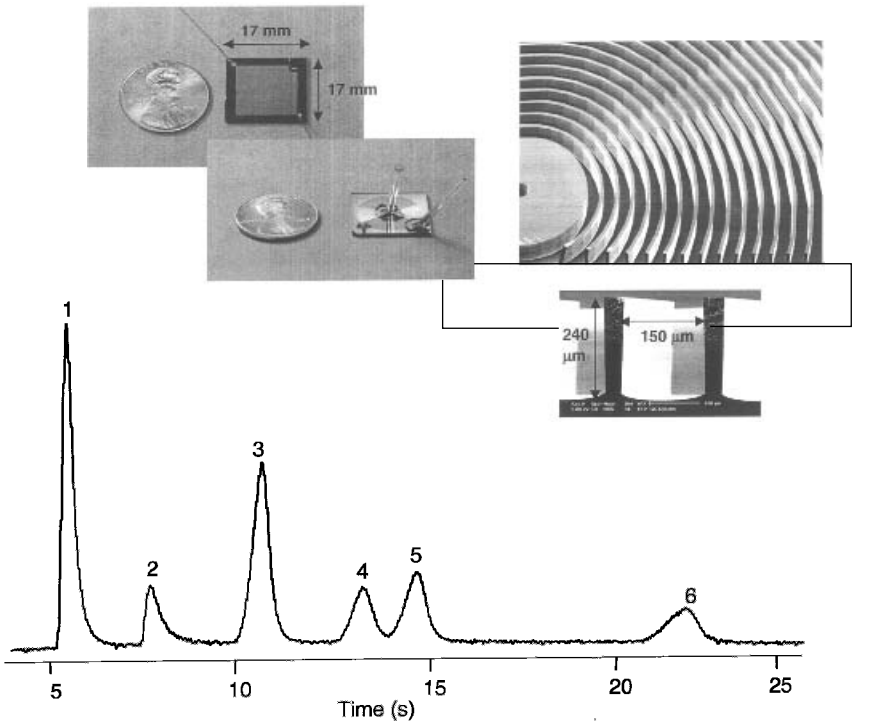

FIGURE 5.26 Microfabricated columns with 0.9-m-long spiral and serpentine channels. Photomicrographs show details of channel shape. The chromatogram was obtained with room air as carrier gas with an outlet pressure of 0.5 atm. The injection plug width was 100 ms. Components are 1, acetone; 2, 2-butanone; 3, benzene; 4, trichloroethylene; 5, 2,5-dimethylfuran; 6, toluene.

rectangular spiral with carrier-gas (air) flow reversing direction at the center of the spiral. The substrate measures 3.2 cm on a side. The etched columns were coated with nonpolar dimethylpolysiloxane. The coating thickness is uncertain but is probably in the 1–2 μm range.

The chromatograms were obtained using room air as carrier gas with a photoionization detector. A vacuum pump was used to obtain a column outlet pressure of 0.5 atm. Room air is continuously pulled through the column. A stepper-motor-controlled gas valve inlet (see Section 5.8.1) was used to inject 100-ms-wide sample vapor plugs at atmospheric pressure. The chromatogram in Figure 5.26 was obtained isothermally at 30°C. The separation is complete in less than 25 s. The chromatogram in Figure 5.27 was obtained with an initial temperature of 30°C and a temperature-programming rate of 60°C/min beginning at the time of injection. Column heating was obtained with a thin-film ribbon resistance heater placed directly under the silicon substrate. Note that the 15-component mixture is completely separated in less than 160 s.

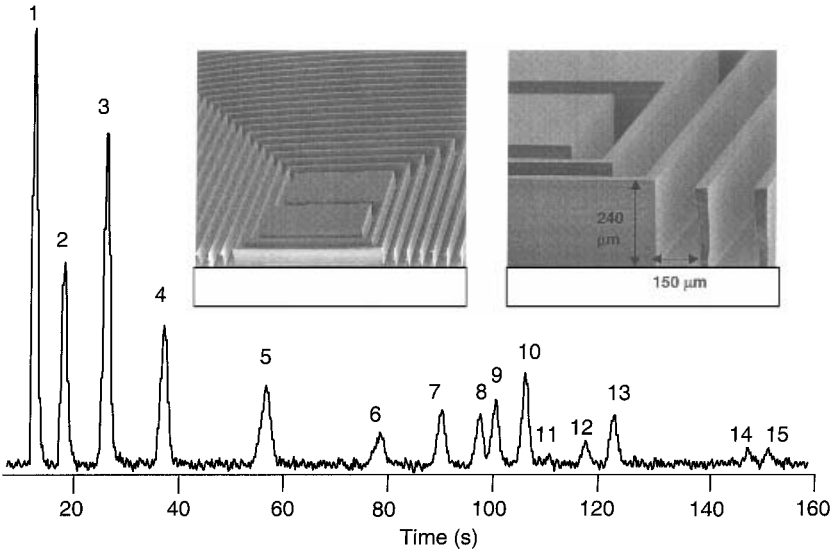

FIGURE 5.27 Microfabricated column showing 3.0-m-long rectangular spiral channel. Photomicrographs show details of channel shape. The chromatogram was obtained with room air as carrier gas with an outlet pressure of 0.5 atm. The injection plug width was 100 ms. Components are 1, acetone; 2, 2-butanone; 3, benzene; 4, 2,5-dimethylfuran; 5, toluene; 6, tetrachloroethylene; 7, chlorobenzene; 8, ethylbenzene; 9, *m*-xylene; 10, styrene; 11, isobutylbenzene; 12, 1,2-dichlorobenzene; 13, heptaldehyde; 14, cumene; 15, α-pinene.

5.15.2 Microfabricated Sensors and Preconcentrators

Two-sensor and four-sensor arrays of chemiresistor detectors have been fabricated on silicon substrates using spray-coated gold nanoparticles with self-assembled organic thiol monolayers as sensing elements. A chromatogram using a *n*-C_8-S-Au sensor is shown in Figure 5.11b. The component selectivity obtained with a two-sensor array using benzene-C_2H_4-S-Au and *n*-C_8-S-Au sensors is shown in Figure 5.28 (30). The design of the interdigital electrodes used with the two-sensor array also is shown. The bar graphs show the relative sensor responses based on peak area measurement for a typical set of target compounds. The large differences in the response patterns of the two sensors for the different compounds provide the basis for component identification based on pattern recognition methods.

Microfabricated versions of the multibed preconcentrator shown in Figure 5.10 are under development. Examples are shown in the photomicrographs of Figure 5.29. The deep-etched silicon structures hold beads of the adsorbent materials. After sample collection, the silicon structures are resistively heated to inject the sample into a microfabricated column. These MEMS preconcentrators are designed to quantitatively collect 30–50 volatile target compounds from 250–500-cm^3 air samples. No performance data are available at this time.

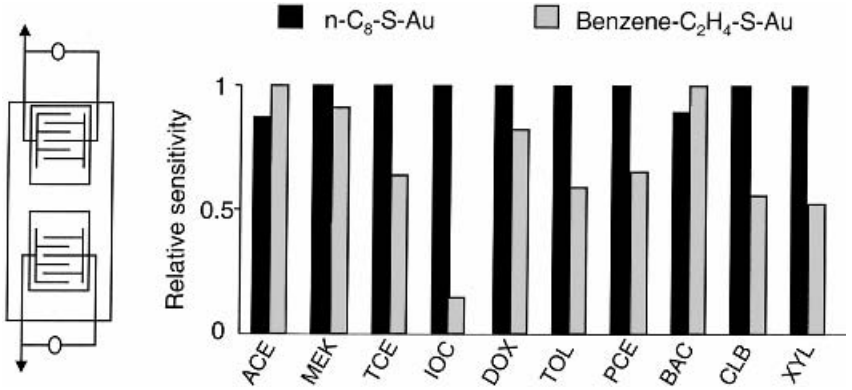

FIGURE 5.28 Selectivity of chemiresistor sensors. Arrangement of interdigital electrodes is shown at left. ACE, acetone; MEK, 2-butanone; TCE, trichloroethylene; IOC, isooctane; DOX, 1,4-dioxane; TOL, toluene; PCE, perchloroethylene; BAC, *n*-butyl acetate; CLB, chlorobenzene; XYL, *m*-xylene.

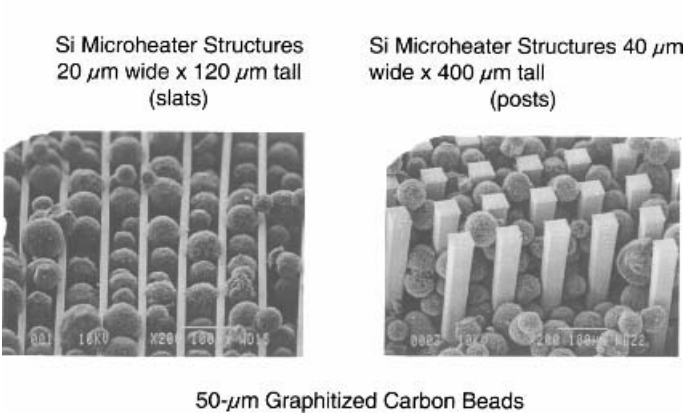

FIGURE 5.29 Photomicrographs of microfabricated preconcentrator using deep-etched silicon slats or posts to hold carbon beads and to resistively heat beads for sample injection.

5.15.3 Complete MEMS GC

A diagram of the complete microfabricated GC instrument for air monitoring is shown in Figure 5.30. Vacuum outlet GC will be used with ambient air as carrier gas and an outlet pressure of 0.5 atm. A prototype instrument using MEMS components is scheduled for completion in 2004. A multibed, sorption-based preconcentrator and injector will be used to collect organic vapors from 250-mL air samples. During sample collection, the flow through the preconcentrator is from top to bottom as shown in the figure. Direct connection to the microfabricated vacuum pump provides for relatively high sampling flowrates (25 mL/min)

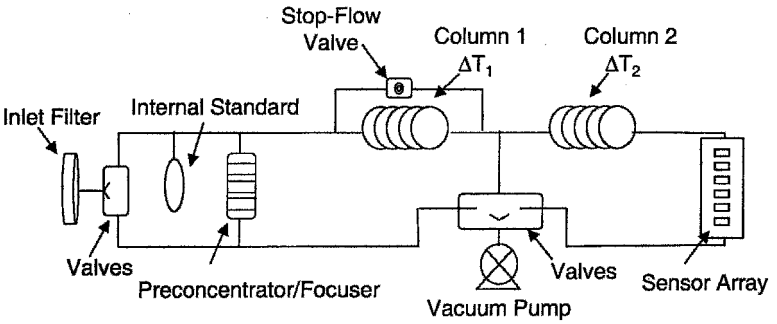

FIGURE 5.30 Diagram of autonomous microfabricated GC for air monitoring. Vacuum outlet GC with ambient air as carrier gas is used to eliminate the need for compressed gases. A dual-column ensemble consisting of two 3.0-m-long columns with independent temperature control and stop-flow operation is used for selectivity enhancement, and a chemiresistor sensor array is used for vapor identification.

during sample collection. After sample collection, microvalves are used to change the flow direction through the preconcentrator. The device is then heated to inject the sample into the first microfabricated column.

The dual-column ensemble will consist of two 3.0-m-long microfabricated columns, one using a nonpolar dimethyl polysiloxane stationary phase (see Figure 5.27) and the other using a moderately polar trifluoropropylmethyl polysiloxane stationary phase. The column ensemble can be operated in the stop-flow mode by the use of a valve between the preconcentrator and the column junction point. Detection is provided by an array of microfabricated chemiresistor sensors, each having a different selectivity. The pattern of responses from the various sensors will be used for vapor recognition and the quantitative analysis of overlapping peaks.

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PART II

Techniques and Instrumentation

Books must follow sciences, and not sciences books.

—Proposition touching Amendment of Laws

CHAPTER SIX

Detectors in Modern Gas Chromatography

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6.1 INTRODUCTION

The detection system in gas chromatography (GC), as in other chromatographic techniques, provides the response signal for the chemical compounds separated by the chromatographic column. A flow of finite amounts of chemical entities arrives at the detector in discrete bands; in GC, these entities are in the gas phase.

These bands have a relatively short residence time in the detector, and therefore, the detector must respond to the presence of the flowing chromatographic solute very fast, in some instances in less than one second. The signal response is characteristic of a physical or chemical property of the chemical compounds being monitored by the detector. There is a variety of detection systems for GC and one must select the appropriate one for the particular application at hand. The detector is typically used to quantify the known components of a sample mixture. In other cases, the primary purpose of the detector is to provide chemical information that would lead to the proper identification of a compound. For either situation, however, it is important to understand the detection mechanism and the experimental parameters affecting the detector's response in order to obtain accurate and reliable results for a correct interpretation of the experimental data. In order to select the appropriate detection system in GC, one must be familiar with the different parameters to measure the performance of a gas chromatographic detector. Many detection systems for GC have been reported in the literature. In this chapter, however, we survey just the most commonly used chromatographic detectors for GC, including their operating principles, design, and performance characteristics.

6.2 GENERAL ASPECTS

6.2.1 Noise Characteristics

Any perturbation in the detector signal that is not related to the eluted sample peak is detector *noise*, which can be caused by several experimental conditions, such as temperature changes, contaminated carrier gas, fluctuation in gas flowrate, a dirty detector cell or jet, column bleed, line voltage fluctuations, and defective electronics. Noise is a very important detector characteristic since it can ultimately dictate the detectability of a given compound, as the signal corresponding to the compound must rise above the noise. Consider the chromatogram illustrated in Figure 6.1. Three characteristic parameters are illustrated in the figure: peak height (h), peak width at half-height (w_h), and noise (N). These are measured in any convenient units, most typically in millivolts. The peak height is measured from the peak base to its maximum. With the digital acquisition of chromatographic data by computers, the statistical moments of the peak profile can be obtained by direct integration, which allows an easy and rapid way to obtain chromatographic parameters (1–3). The peak width at the base (w_b) or at half-height (w_h) can be obtained from a multiple of the peak variance, the second moment. There are other methods to obtain w_b: (1) twice the peak width at half-height, (2) the base of a triangle that most closely matches the peak shape, and (3) twice the ratio of peak area (A) to peak height ($2A/h$). The first method is perhaps the simplest of all; however, it is not as accurate as using the peak variance or the ratio $2A/h$.

The noise is the average peak-to-peak measurement between the highest and lowest excursion of the baseline over a period of time. There are different types

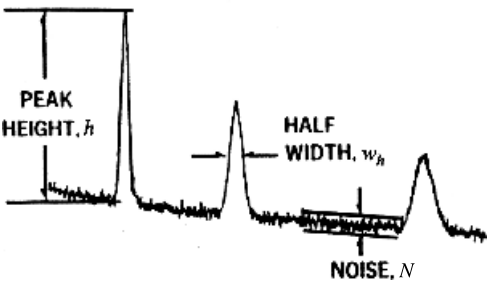

FIGURE 6.1 Portion of a chromatogram indicating peak height, peak width at half-height, and noise (reprinted with permission from previous edition; copyright 1995 John Wiley and Sons, Inc.)

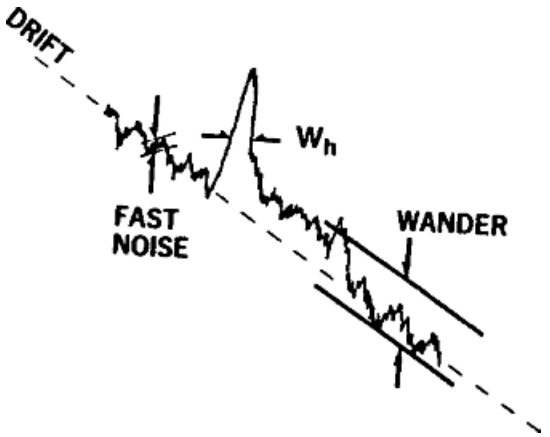

FIGURE 6.2 Portion of a chromatogram illustrating different types of noise (reprinted with permission from previous edition; copyright 1995 John Wiley and Sons, Inc.)

of noise. One can encounter short-term noise, long-term noise, and drift. These are all disturbances to the baseline of a chromatogram and are represented in Figure 6.2. The *short-term noise* (sometime known as "fast noise") consists of high-frequency perturbations to the baseline. The frequency of this type of noise is higher than the peak of interest and is usually eliminated by means of an appropriate noise filter (long-pass filter). *Long-term noise*, on the other hand, is manifested as baseline perturbations that are of lower or similar frequencies than that of the peak of interest. This type of noise is difficult to differentiate from a chromatographic peak of similar amplitude. In such instances, the noise cannot be removed without removing the peak of interest as well. Long-term noise is most likely caused by instability of detector components and/or fluctuations of ambient conditions (e.g., cyclical changes in ambient conditions and line voltage). *Drift* pertains to variations in the baseline that are very slow and constant over time.

It can be expressed as the average slope of the noise envelope, typically in millivolts or microvolts per hour. A chromatographic peak is not obscured by drift; however, detectors operating under severe drift conditions would require frequent adjustment of the baseline. More often, drift occurs by changes in the effluent of the column (e.g., bleeding of the column, changes in supporting or makeup-gas flowrate) and "bakeout" of contaminants components within the detector.

A measurement of noise will include the maximum amplitude of the combined short- and long-term noise; drift is typically ignored. At a significant noise level, it is recommended to measure noise over several peak widths in the same units as the peak height, as represented in Figure 6.2. Modern chromatographic data acquisition systems can measure noise automatically, and can easily display it on a computer screen.

A parameter frequently used to characterize a detector is the *signal-to-noise* ratio (S/N). The signal-to-noise ratio is indicative of the probability that a particular peak, in a noisy baseline, represents the signal from an analyte. The signal-to-noise ratio of 2 indicates a 95% probability of the signal being a sample peak. A signal-to-noise ratio of 2.65 increases the probability to 99%. The S/N is estimated using measurements of the peak height relative to the baseline noise, including both short- and long-term noise. The S/N is relatively easy to measure; Figure 6.3a illustrates the important measurements from a chromatogram. First,

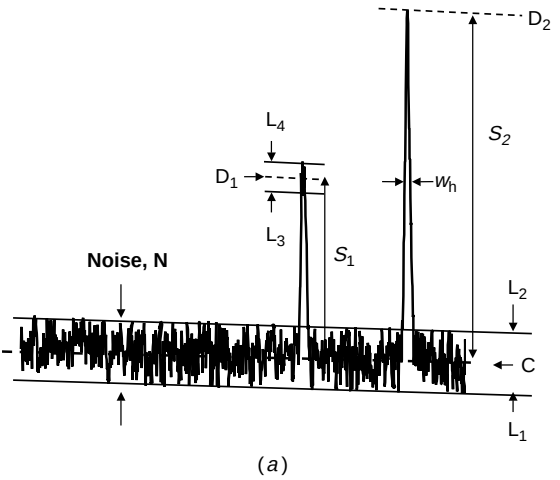

FIGURE 6.3 (a) Illustration of measurable parameters in a chromatogram used for a signal-to-noise calculation, with the noise envelope defined between L_1 and L_2 and identifying the mean noise (i.e., C) between the positive and negative peak excursions. Then, the signal peak height (S_2) is measured from the mean noise C to the peak apex D_2, and the S/N is calculated. For a noisy signal (e.g., S_1), the peak height should be measured from the estimated mean of the apex signal; for S_1, this is D_1 between L_3 and L_4. (b) Illustration of measurable peaks in a chromatogram used for a signal-to-noise calculation.

(*b*)

FIGURE 6.3 (*Continued*)

one must define the noise envelope (between L_1 and L_2) and identify the mean noise (i.e., C) of the positive and negative peak excursions. Then, the signal peak height (S_2) is measured from the mean noise C to the peak apex D_2, and the S/N is calculated. For a noisy signal (e.g., S_1), the peak height should be measured from the estimated mean of the apex signal; for S_1, this is D_1 between L_3 and L_4 in Figure 6.3a. An interactive computer graphical method can also be used to calculate S/N (4). Figure 6.3b shows a chromatogram with two peaks recognizable above the noisy baseline with S/N of $\sim$2 for the first peak and about 2.8 for the second; the dashed lines indicate the magnitude of the noise.

6.2.2 Sensitivity

Detectors can be conveniently divided into two major groups: *mass flow detectors*, which respond to the mass of sample component reaching the detector per unit time (e.g., ng/s), and *concentration-sensitive* detectors, which provide an output that is directly proportional to the concentration of a sample component in the mobile phase (e.g., ng/mL). *Sensitivity* (S) of a detection system is defined as the change in detector signal with a change in mass or concentration of the eluted solute. In essence, sensitivity is the slope of the calibration plot, which is a graph of the detector response versus analyte mass or analyte concentration for a given component. Sensitivity has the units of detector response per unit mass or concentration of analyte (e.g., mV/pg). The term sensitivity has been used to compare different detection systems with the meaning *minimum detectable level*, but this can lead to confusion that can be avoided. We recommend the use of *limit of detection* (LOD) to refer to the minimum detectable level, and reserve the term *sensitivity* for the slope of the linear calibration plot.

6.2.3 Limit of Detection

The *limit of detection* (LOD) or *minimum detectable level* (MDL) refers to the quantity or concentration of solute, which generates a peak height (or peak area)

corresponding to a S/N of 2; although recently a S/N of 3 is commonly used. This is the minimum mass (or concentration) flow of the substance of interest in the mobile phase for which the detector can give a response with certain probability (>99% for a S/N of 3) that the signal represents a sample peak. The LOD can be determined from the measured sensitivity S the noise N and the peak width at half-height, w_h in seconds, as expressed in the following equation for a signal-to-noise ratio of 3:

$$\text{LOD} = \frac{3N}{Sw_h} \tag{6.1}$$

Alternatively, the LOD can be obtained by measuring the noise level, the signal (peak height or peak area), and the peak width at half-height for the component of interest in a given chromatogram. In Figure 6.3a, for example, if the peak signal for the second peak corresponds to 750 pg and w_h is 5 s, the mass flowrate would be 150 pg/s (750 pg/5 s). If the S/N for this quantity is equal to 5.5, then the LOD corresponding to a S/N of 3 would be 82 pg/s. For concentration sensitive detectors, the flowrate will affect the quantity reaching the detector per unit time, leading to an apparent improvement in the LOD. However, in terms of the concentration of the sample in mass per unit volume (e.g., ng/mL), the LOD does not change.

It is also common to see the LOD reported in terms of mol/sec or mol/mL for a given component. In the case of detectors responding specifically to a given element (or heteroatom), where the LOD is reported as g/s or g/mL, the *mass* refers to the particular element monitored by the detector. For instance, azobenzene is 15% nitrogen by weight and if a detector responding selectively to nitrogen has an LOD of 10 pg/s for azobenzene, this corresponds to 1.5 pg/s for nitrogen. To refer LOD for a particular element X, the element is specified in the LOD as g(X)/s or g(X)/mL (see Table 6.1).

6.2.4 Dynamic Range

The *dynamic range* of a chromatographic detector is defined as the range of concentration or mass over which the detector exhibits an incremental signal response with an incremental change in concentration or mass of solute reaching the detector. The LOD for a given compound is the lower limit of the dynamic range, while the upper limit of the dynamic range represents no observable increase in signal with an increase in injected solute quantity. The most significant region of the dynamic range is the *linear range*, which denotes the range of concentration or mass flow over which the *sensitivity* of the detector (i.e., slope of the calibration plot) is constant over a specified variation, commonly ±5%. These parameters are illustrated in Figure 6.4. Because linearity can extend over several orders of magnitude, an alternative path to establish the linear dynamic range utilizes the detector's response factor for a probe solute (see Section 6.2.5) as illustrated in Figure 6.5 (5). In such a case, a plot of the response factor versus the mass flowrate defines the linear dynamic range for a particular compound.

TABLE 6.1 Summary of Several Typical Characteristics for Common GC Detectors

Detector	Selectivity	LOD	Linear Range
Thermal conductivity detector (TCD)	Responds if thermal conductivity is different from carrier gas (Universal)	1 ng/mL	10^5
Flame ionization detector (FID)	Organic compounds	1 pg(C)/s	10^7
Electron-capture detector (ECD)	Electron-capturing compounds such as halogens	10 fg/s (lindane)	10^4
Nitrogen–phosphorus detector (NPD) or thermionic detector	N- and P-containing compounds	1 pg N/s 0.5 pg P/s	10^4
Flame photometric detector (FPD)	P- and S-containing compounds	50 pg S/s 2 pg P/s	10^3 10^4
Photoionization detector (PID)	Aromatics	5 pg C/s	10^7
Electrolytic (Hall) conductivity detector (ELCD)	Halogens and S	1 pg Cl/s 5 pg S/s	10^6 10^4
Atomic emission detector (AED)	Element selective	0.1–50 pg/s depending on element	10^4

The LOD and the ±5% limits are also indicated on the graph. The magnitude of the linear range of a detector is dependent on the test substance used; hence, when reporting the linear range, one must specify the test substance used and the LOD for the substance. One must also realize that the linear range for a given detector specified by a manufacturer may have been determined under optimal conditions; however, under a more practical setting the range attainable can depend on chromatographic parameters such as column temperature, detector temperature, and flowrate. In Figure 6.5, one can also observe a portion of the dynamic range that is not linear. An ideal gas chromatographic detector would have low LOD and a large linear dynamic range (five to six orders of magnitude).

6.2.5 Response Factor

The *response factor* is the ratio of the signal-to-sample size, and is used for more accurate quantitative analysis with a gas chromatographic detector. The response factor can be defined incorporating either peak area or peak height; both are

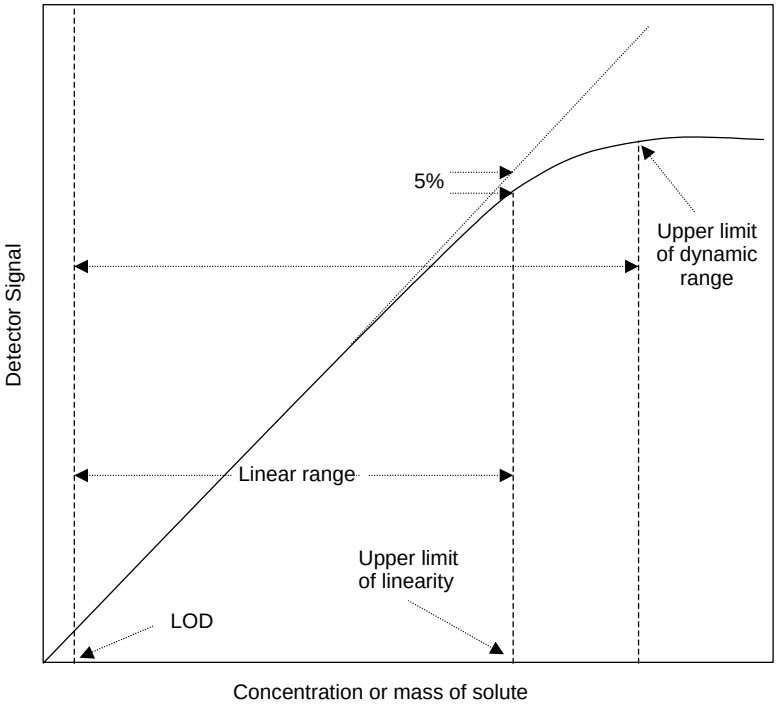

FIGURE 6.4 Graphical method used to illustrate linearity of a detector.

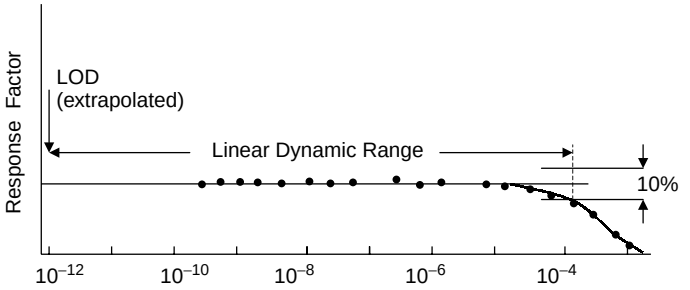

FIGURE 6.5 Method of plotting response factors to demonstrate linearity (adapted from Reference 5 with permission; published in 1969, Friedr Vieweg and Song).

related through the following equation

$$A = \frac{hw_b}{2} \tag{6.2}$$

where A is the peak area, h is the peak height, and w_b is the peak width at the base. The response factor in terms of mass M injected, can be determined using the

TABLE 6.2 Equations to Determine Response Factor in Terms of Mass (M) of Component Injected, Peak Width (w_b), and Flow (F)

	For Peak Height, h	For Peak Area, A
Mass-flow-sensitive detectors	$hw_b/2M$	A/M
Concentration-sensitive detectors	$hw_bF/2M$	AF/M

equations presented Table 6.2 for the mass sensitive and concentration sensitive detectors. To avoid confusion caused by the use of different instruments and temperature conditions, it is recommended to express the peak height or area in terms of the actual detector output and retention factor (k). For example, the peak height (or area) for the FID is expressed in terms of amperes [or amperes-seconds ($A \cdot s$) for area].

6.2.6 Selectivity

For a particular analysis, the choice of one gas chromatographic detector over another depends on the abovementioned parameters (LOD, sensitivity, linear range, selectivity in response, etc.) and the ability of the detector to respond to the solutes of interest. With this in mind, gas chromatographic detectors can be generally classified as selective or universal detectors. A *selective* detector responds to compounds containing a specific heteroatom while a *universal* detector responds to any solute eluting from the chromatographic column. For example, the flame ionization detector (FID) is commonly used in the petroleum analysis, as it responds to hydrocarbons of interest that can be present at very low levels. The FID responds selectively to hydrocarbons; hence, the FID is a *selective* detector for hydrocarbons. In the case of analyzing natural gas, several components of interest (e.g., N_2 and CO) have little or no response in the FID. For this application, the thermal conductivity detector (TCD) is a more appropriate choice since it is capable of responding to all the components of the gas sample. Here one must sacrifice low limits of detection for universality. The TCD is a *universal* detector, since it responds to all constituents in the sample; the selective detectors, in contrast, respond to a limited number of compounds. Among selective detectors, we find element-specific detectors, which respond specifically to a particular element of interest (e.g., microwave plasma emission detector).

Figure 6.6 illustrates the practicality of using a selective detector in a particular analysis (6). The figure shows chromatograms of a mixture containing 10 pesticide standards added to milk, using different detection systems. The FID responds to every organic component in the extract (Figure 6.6a). One can see that the compounds of interest (i.e., the pesticides) cannot be resolved from the background. The FID cannot be used unless a considerable amount of sample treatment is performed to eliminate the interferences. The use of a selective detector with low or no response to the interfering materials becomes more practical.

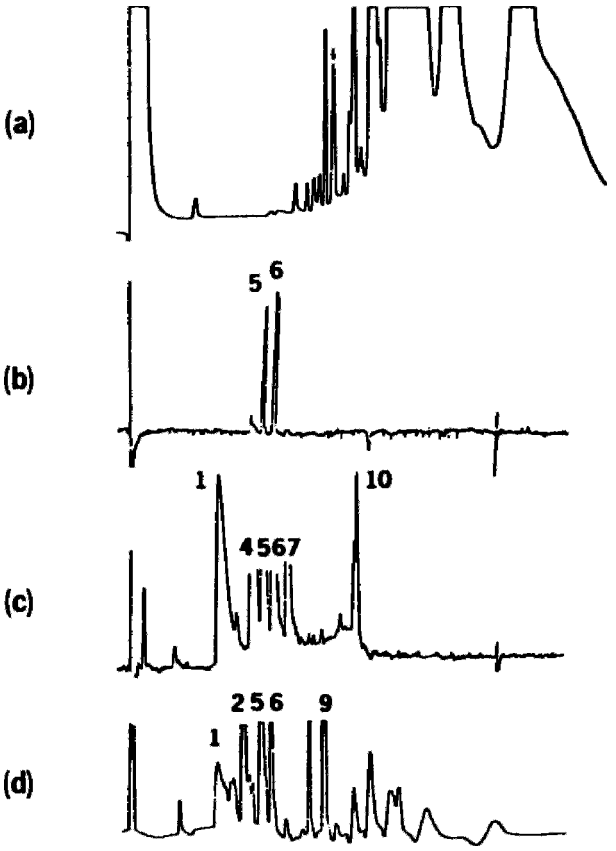

FIGURE 6.6 Chromatograms of a mixture of 10 pesticide standards, extracted from milk, and run on various detectors: (a) flame ionization detector; (b) flame photometric detector, sulfur mode; (c) flame photometric detector; (d) electron-capture detector. (Adapted from Reference 6 with permission. Copyright 1975 American Chemical Society.)

In the other chromatograms in Figure 6.6, other selective detectors are used to analyze the sample. The selective detectors allow analysis of the pesticides that were previously unresolved from the background. Here, one can appreciate how small amounts of a component can be detected in a relatively large complex matrix by using selective detectors. The selectivity of a given compound over another is determined by obtaining the ratio of their sensitivities. In order to find small components in a large interfering matrix, a selectivity value of less than three orders of magnitude is of little value.

When using selective detectors, one must consider the possible change in the response factor for a particular component due to the presence of the background matrix. For example, a selective detector for sulfur and phosphorus-containing compounds is the flame photometric detector, but it shows a diminished response

for sulfur-containing compounds if a large quantity of hydrocarbons coelute with the sulfur compound. In contrast, some modern electron capture detectors show an enhancement in signal for low-level pesticides with a small increase in the amount of column bleed. It is important to run appropriate controls to prevent errors due to the abovementioned phenomena. Sample blanks, standards, and sample spiking must be run in addition to the samples. A *sample blank* contains the matrix of the sample without the solutes of interest. The *standards* include a mixture of the components to be determined in a known concentration. *Spiking* can be performed by adding a known amount of standard to a blank or a previously analyzed sample. The blank provides checks for adequate selectivity; the standards are used for calibration, whereas spiking provides for checks of the response factors by confirming if there are differences between the components in standard mixture and in the presence of the interfering background.

6.2.7 Other Practical Considerations

Although each gas chromatographic detector has specific operating parameters and instructions, several general guidelines apply to all detectors, which are given below. For information on a particular detector, refer to the section below corresponding to the detector in question.

1. In order to avoid temperature variations in the detector, the gas chromatograph should be positioned away from drafts and heating or air-conditioning vents. Locations near poorly insulated outside walls or with direct sunlight must also be avoided.
2. Problems can arise if there are leaks in the gas lines. With a gas leak, air can diffuse into the system. For example, air is particularly detrimental for a conductivity detector and, of course, is detrimental to column performance. One must always check for gas leaks.
3. The chromatographic column should not be conditioned while connected to the detector.
4. To avoid condensation buildup in the detector when starting the gas chromatograph, the oven should be brought to the operating temperature before turning on the detector, since the oven heats more rapidly than the detector.
5. Better thermal stability is obtained if side panels or top covers of the instrument are not removed.
6. The use of scrubbers, moisture traps, and the like are recommended to remove contaminants from any supply of detector gases. They, however, must be replaced when exhausted; otherwise, they will add impurities instead of removing them.
7. When running high-concentration or “dirty” samples, it is a good practice to check the detector exit tubes for condensation and clogging and clean them when appropriate.
8. Leaving the detector at operating temperature overnight can facilitate achieving a stable temperature for the next morning.

6.3 THERMAL CONDUCTIVITY DETECTOR

6.3.1 Introduction

The thermal conductivity detector (TCD) is a universal, nondestructive detection system. Since thermal conductivity is a bulk physical property, the TCD is also identified as a bulk property detector, because it responds to some difference in the thermal conductivity of the carrier gas caused by the presence of the eluted components. Devices designed to measure thermal conductivity have been described since the 1880s, and in the early 1900s they were used in a variety of applications (e.g., gas analysis in chemical industries) (7). Since it was an established technique, it was a natural step to couple thermal conductivity with GC in early developments of the technique. It took some time to appreciate the advantages of using helium or hydrogen as the carrier gas with the TCD. By the end of the 1950s, the TCD was a mature detection system for gas-solid chromatography. Despite many attempts to improve it, the detectability of the TCD continues to prove problematic for trace determinations. With the development of ionization detection systems, which offered improved detectability, particularly for use with capillary columns, the TCD was replaced by ionization detectors and still remains as the most convenient and inexpensive detector for use in less demanding analyses. The TCD, however, is very useful in the determination of gaseous substances that are difficult to detect by other means, particularly in gas–solid chromatographic analysis using packed columns; for example, in the determinations of substances such as CS_2, COS, H_2S, SO_2, CO, CO_2, NO, and NO_2. The development of flowthrough microflow cells with very small volumes has also allowed the use of the TCD with open tubular capillary columns (8).

6.3.2 Operating Principles

When a material is submitted to temperature difference, conduction of heat takes place from the points of higher temperature to the points of lower temperature. The property of how well a material conducts heat is known as the *thermal conductivity* λ. Consider Figure 6.7, in which two planar surfaces are at two different temperatures (T_1 and T_2) separated by distance x. The heat flow (Q) through the material with thickness x depends on the cross-sectional area (A), temperature difference, and conductivity (λ) as follows:

$$Q = \frac{A(T_1 - T_2)\lambda}{x} \tag{6.3}$$

Adopting a cylindrical configuration, losses other than conduction through the gas are avoided and Equation 6.3 becomes

$$Q = G(T_1 - T_2)\lambda \tag{6.4}$$

where G is a geometry factor that replaces the dimensions A/x. For the TCD the cylindrical geometry is obtained by replacing one of the planar surfaces

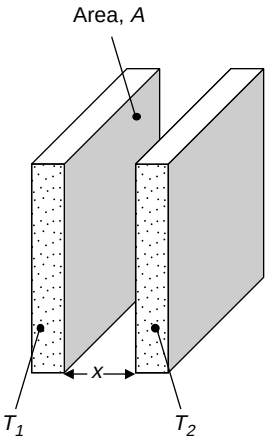

FIGURE 6.7 Parameters involved in thermal conductivity between two surfaces of temperature T_1 and T_2 and cross-sectional area A, separated by a distance x.

in Figure 6.7 with a cylindrical cell at T_2 and the second surface with a wire filament running through the center of the cylindrical cell at T_1 (see Figure 6.8). The thermal conductivity is obtained by supplying heat at a known rate Q and measuring the temperatures of the wire filament at the center and the outside wall of the cylindrical cell. The heat flow is provided by applying an electrical current, I (amperes), through the center wire filament of known electrical resistance, R_f [ohms (Ω)], given by

$$Q = \frac{I^2 R_f}{J} \tag{6.5}$$

where J is Joule's constant (4.183 W/cal·s). Using a wire with a temperature-dependent resistance, one can measure the temperature of the wire according to Equation 6.6, serving a dual purpose:

$$R_f = R_f^0(1 + \alpha T_1) \tag{6.6}$$

In Equation 6.6, α is the temperature coefficient of resistance for the wire filament and R_f^0 is the resistance at the reference temperature of 0°C. T_1 is obtained from Equation 6.6 by knowing the voltage across the wire and utilizing current I to calculate the resistance (at temperature) ($R = V/I$).

For the TCD in GC, the absolute value for λ that can be calculated from the temperatures, heat flow, and geometry of the cell is not important. However, the small changes in λ that are caused by the presence of sample components in the carrier gas and changes in T_1, which can be detected by the change in the resistance of the wire filament, are important:

$$R_f = \alpha R_f^0 \Delta T_1 \tag{6.7}$$

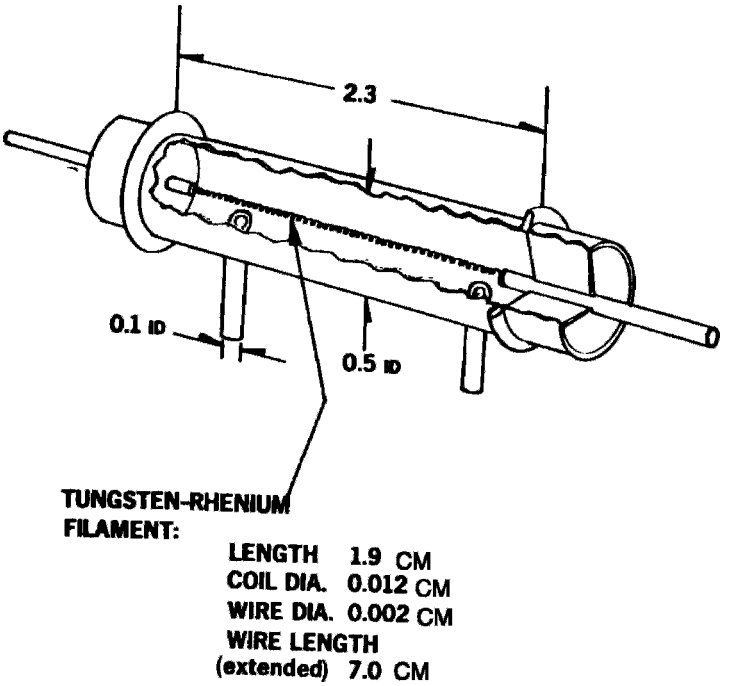

FIGURE 6.8 Geometry of a typical TCD cell. The supports for the axial filament are insulated from the stainless-steel body. Dimensions are in centimeters. (Reprinted with permission from previous edition. Copyright 1995 John Wiley and Sons, Inc.)

$$\Delta T_1 = \frac{\Delta\lambda(T_1 - T_2)}{\lambda} \tag{6.8}$$

Combining the equations and rearranging

$$\Delta R_f = \frac{-\alpha R_f^0 \Delta\lambda(T_1 - T_2)}{\lambda} \tag{6.9}$$

The sensitivity for changes in λ is proportional to the temperature difference across the cell. The wire filament, however, has a limit in temperature to which it can be submitted. For most commercially available TCDs using a hot wire filament, 450°C is a practical upper limit for continuous operation that can be increased to about 500°C for short operation periods. The upper temperature limit is imposed by the oxidation of the wire at high temperatures by trace oxygen in the carrier gas. If the sample contains components at relatively high concentrations, other reactions are possible. At the maximum wire temperature, the LOD can be improved by decreasing the temperature of the detector T_2. In practice, the best detectability is obtained by applying the maximum allowed current to the wire filament, without burning it out, and setting the detector block temperature to

the lowest setting possible without allowing the eluted compounds to condense inside the cell. These two steps maximize ΔT.

In addition to the thermal conductivity of the carrier gas, heat from the wire filament can be dissipated through thermal radiation, thermal conductivity through the end of the filament (end losses), and convection. Thermal radiation depends on the surface area, temperature, and quality of the filament. For a detector with the characteristics of those shown in Figure 6.8 and Table 6.3 (see Section 6.3.3), an estimate of the power loss through thermal radiation is approximately 15 mW. A few percent of the power can be transferred through radiation at the highest temperature. Although the temperature of the filament is constant over its length, heat is lost through the end of the filament because, the temperature at the end drops to the temperature of the cell body. The loss is proportional to the difference in temperature between the filament and the cell body; for the example cited in Figure 6.8 and Table 6.3 the heat loss is about 45 mW. For the high-conductivity carrier gases and small diameter filaments in use today, this heat loss is negligible.

The heat loss due to mass flow convection of the gas is the product of the specific heat of the gas, gas mass flowrates, and the difference in temperature between the gas exiting the cell and the detector temperature. This contributes to the noise and drift of the detector. For the geometry considered in Figure 6.8, the temperature of the gas exceeds the temperature of the block by only 2.4% when there is a 200°C difference across the cell, corresponding to a heat loss of approximately 7 mW, assuming a uniform axial flow.

The heat transfer effects considered above for the particular cell in Figure 6.8 and Table 6.3 are shown in Table 6.4. With the exception of the thermal

TABLE 6.3 Typical Operating Conditions

Temperature	
Body of detector	150°C
Filaments	350°C
Filaments	
Material	Tungsten–rhenium
Temperature coefficient, α	0.0033/°C
Resistance at 0°C, R_f^0	25 Ω
Resistance at 350°C, R_f	55 Ω
Electrical (four-element bridge)	
Current	0.3 A
Voltage	16.5 V
Power (for each filament)	4.95 W
Current	0.15 A
Voltage	8.25 V
Power	1.24 W
Carrier gas	Helium
Flowrate	1.0 mL/s
Thermal conductivity, λ (at 150°C)	4.4×10^{-4} cal/s·cm·°C

Source: Reference 7.

conductivity of the gas, the effects contribute just a few percent to the heat transfer. Using nitrogen as the carrier gas, the thermal conductivity term would be 6 times lower, making the other terms comparatively more significant.

6.3.3 Detector Design

The TCD is commonly operated using helium or hydrogen as the carrier gas. The thermal conductivity of these gases is higher than virtually all solutes. When other gases are used as the carrier gas (i.e., N_2), they present several problems: (1) some components yield positive peaks, and others yield negative peaks; (2) response factors are temperature dependent with unexpected behavior, and linearity is poor; (3) the LOD is usually one order of magnitude worse; and (4) in some cases, W-shaped peaks are observed as a result of changes in conductivity with changes in sample concentration.

Several different detector designs have appeared for the TCD (9). The most general-purpose TCD cell geometry is illustrated in Figure 6.8. One common variant of this design supports the filament from two posts, both of which are mounted on the same face of the cavity. Coiled filaments are used instead of straight wire to obtain the maximum resistance. Most filaments are made of platinum, tungsten, nickel, or alloys of these with other metals, such as rhenium or iridium. The resistivities and temperature coefficients (α) of these materials are similar; therefore, the choice of filament material depends on mechanical strength and chemical inertness. Typical operating conditions for the filament are listed in Table 6.4.

The thermal conductivity cell is extremely sensitive to variations of physical variables, such as temperature fluctuations, affecting the performance of the detector. Some of these variations can be cancelled out by using two cells (Figure 6.9), one to detect the sample and the other used as a reference. In such a design, a Wheatstone bridge–type circuit is used to balance the resistance, R_3 of the sample cell against the resistance R_4 of the reference cell. The remainder of the circuit is composed of two fixed resistors (R_1 and R_2). All resistors have the same value if no substance is eluted from the column; hence, no voltage difference is measured. As a substance elutes from the column and enters the cell, the

TABLE 6.4 Summary of Heat Transfer Effects with a TCD

Effect	Heat Transfer (mW)
Thermal conductivity (G = 3.08)	1130
Radiation	15
End losses	45
Mass flow	7
Free convection	Negligible
Total (measured)	1240

Source: Data obtained from Reference 7.

resistance R_3 decreases, the Wheatstone bridge becomes unbalanced and a voltage difference is measured. A fluctuation in room temperature would affect the analytical and reference cell similarly, and no significant voltage change would be measured. This design can compensate for column bleed by using the same flow through the analytical and reference cells, and have the flow through the reference cell through an identical column. Such an arrangement is useful with temperature programming.

To overcome the effect of temperature on the conventional resistors in the design of Figure 6.9, one can control the temperature of the resistors or use resistors at very low temperatures. The four-filament configuration shown in Figure 6.10 replaces each fixed resistor with a filament cell for improved stability.

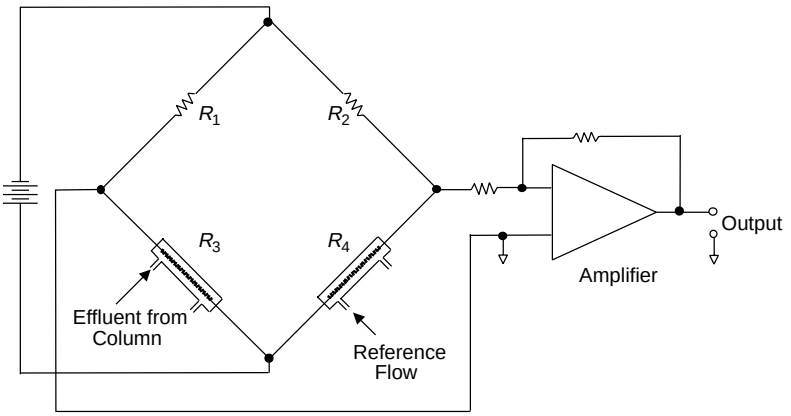

FIGURE 6.9 Bridge circuit used in a two-cell detector. The reference cell R_4 compensates for drift in the analytical cell R_3 due to flow temperature fluctuations.

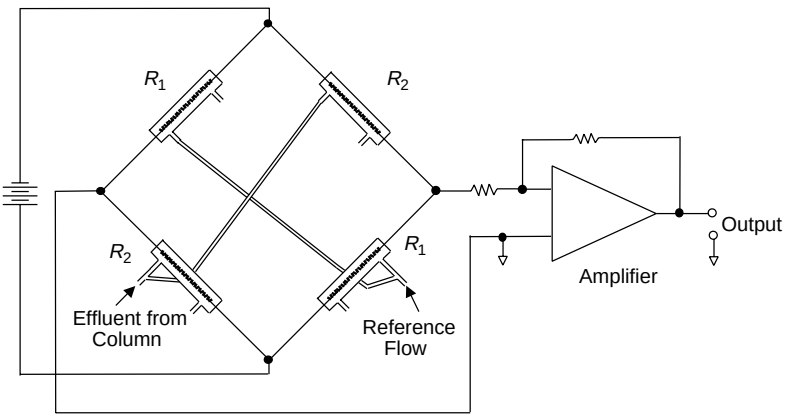

FIGURE 6.10 Bridge circuit used in a four-cell detector. This approach gives twice the response shown in Figure 6.9.

The flow from the analytical column enters two matched resistors of resistance R_2, while the reference flow of the reference enters two matched reference cells of resistance R_1. In some detector designs, the matched resistors are simply the incorporation of two filaments mounted inside the same cell cavity. For such a design, the response factor is increased by a factor of 2 since the two filaments are contributing to the change in signal. Although not shown in the figures, variable resistors are also included in the bridge circuit to null the output voltage before running a sample and are controlled by fine and coarse adjustments. This is necessary since the four cells do not typically match exactly.

The electrical requirements for the TCD are much simpler than those for other gas chromatographic detectors. The mechanical requirements, however, are usually demanding, particularly thermal control. It is extremely important to control the temperature of the detector very well. To accomplish this, the cells of the TCD are mounted closely together, embedded in a metal block, with the entire assembly meticulously insulated. Often, the temperature control of the circuit provides better thermal stability than the chromatographic oven. Insulation of the detector prevents heat transfer by thermal conduction from the chromatographic oven. If heat is transferred through the flowing carrier gas, variations in the gas flow will likely be the source of noise and drift.

The filaments of the detector must be matched, not just electrically but also mechanically, due to the impact of the geometry factor. The cells must be gastight, even at high temperature, to avoid diffusion of air into the cell, which will result in drift and noise and ultimately contributes to a reduced lifetime of a filament. Noise can also be caused by electrical leakage from the filaments to the detector body; this, however, is prevented by insulating the filament mounts with high-density ceramics.

Thermistors have also been used in thermal conductivity detectors instead of filaments. These small metal oxide beads are temperature-sensitive resistors and have been implemented in the TCD since the 1950s. They do have several advantageous characteristics. Thermistors have a large negative temperature coefficient of resistance. Since they can be made very small (e.g., 250 μm diameter), small volume cells can be fabricated (e.g., 50 μL). Thermistors are almost inert to oxidizing conditions because they are metal oxide glass. On the other hand, they are fragile and sensitive to reducing conditions and as a result, need an inert coating to alleviate the problem. The major problem with a thermistor as a sensing element is the fact that detectability decreases rapidly as the temperature of the detector rises above 50°C. In addition, the operating conditions of the detector are more difficult to set. The thermistors are used mostly when small detector volumes and fast responses are required, as in the case of capillary columns and for gaseous samples that can be run at low temperatures.

Another design of the TCD is known as the *modulated detector*. The flow of gases in the detector cell is illustrated in Figure 6.11. The flow of the analytical column effluent is alternated with the reference flow at a rate of 10 times per second, which is faster than any changes due to the thermal fluctuations.

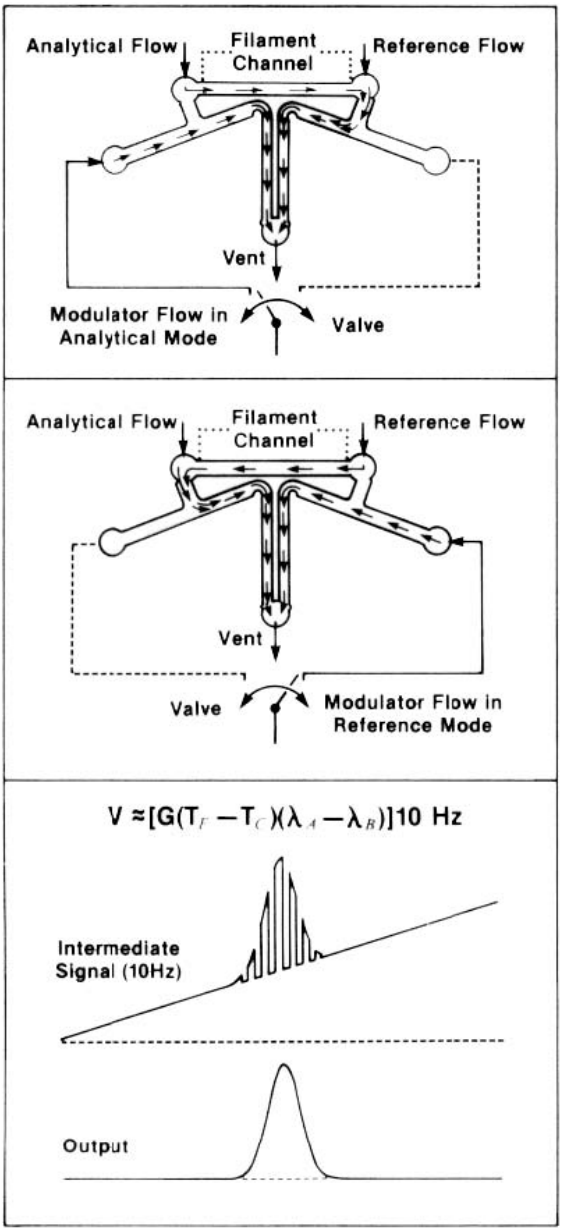

FIGURE 6.11 Diagram of a commercial modulated TCD that uses only one filament cell. The analytical and reference gas flows are switched at a rate of 10 Hz. This design results in a detector with very low drift. This assembly is further insulated on all sides mounted in a metal enclosure. (Reprinted with permission from previous edition. Copyright 1995 John Wiley and Sons, Inc.)

This facilitates ease of startup and reduces baseline drift during temperature programming.

6.3.4 Performance Characteristics

6.3.4.1 Response

The ease of quantitative analysis is one of the best features of TCDs operated with helium carrier gas. Relative response factors generated with an internal standard are independent of (1) cell and filament temperatures, (2) type of detector (wire filament or thermistor), (3) detector current, (4) helium gas flowrate, and (5) sample concentration. Furthermore, only slight changes in the relative retention factors are observed in a series of homologous compounds. The first systematic study of TCD responses in helium was performed by Rosie and Grob (10), a summary is presented in References 9 and 10. As a rule of thumb, all the compounds have a weight response close to that of isooctane, with three major exceptions: (1) compounds containing heavy atoms usually have low response factors, (2) halogenated compounds also tend to have low response factors, and (3) very light compounds (molecular weight <35) tend to have high response factors. Of the compounds reported in References 9 and 10 (171 compounds), for which there are response factors and excluding the abovementioned exceptions, 88% are within 20% of the response for isooctane and 96% are within 30%. Response factors have also been tabulated using hydrogen and nitrogen as carrier gas (11,12).

6.3.4.2 Noise, Detection Limits, and Linearity

The noise in a TCD is subject to many extraneous effects that influence the noise and drift in the detector response. For example, some of the changes in detector output due to the sample for the conditions of Figure 6.8 and Table 6.4 are listed in Table 6.5. A well-designed/operated TCD, however, is capable of noise levels as low as 2 μV. The TCD has detectability in the range of 10^{-6}–10^{-8} g/mL in carrier gas. For the conditions of Table 6.4 (a response factor of 7000 mV·mL/mg), LOD of 1 ng/mL for a signal that is 3 times the noise level can be achieved. This relatively low value is a limitation since other detectors can exceed this by a factor of 10^4–10^7. The linear response of the TCD is about four to five orders of magnitude.

TABLE 6.5 Changes in Detector Output due to Sample

Effect	Change
Sample concentration at peak maximum	0.02 mg/mL
Thermal conductivity	$4.4–4.14 \times 10^{-4}$ cal/s·cm·°C
Filament temperature	350–362°C
Filament resistance	55–56 Ω
Voltage across filament 8.25–8.39 V	
Detector response factor	7000 mV·mL/mg

6.3.5 Other Practical Considerations

1. The carrier gas must be flowing into the detector whenever the voltage is applied to the bridge current to avoid excessively high filament temperature. Some gas chromatographs may incorporate a pressure transducer to turn off the filament current if the carrier flow is interrupted.
2. The filament current must also be turned off when changing columns, septa, or gas cylinders, as these tasks may introduce air into the carrier line.
3. Shifts in the baseline after elution of a large peak may be indicative of a change in the resistance of the sample filament, which can be caused by the presence of an oxidizing, halogenated, or strong reducing compound. If this occurs, the changes can be equalized by periodically reversing the sample and reference sides of the detector. In addition, gold-coated filaments are available that resist oxidation.
4. The filament life is extended by turning off the current when the detector is not in use. However, leaving the current on for extended periods of time (along with the oven temperature) gives the highest detector stability in the morning.
5. For temperature programming, set the detector temperature slightly above the highest column temperature. Higher setpoints sacrifice detectabilities.
6. At high operating filament currents, better detectability is obtained, while at low filament currents the life of the filament is extended. Following the instructions provided in the operating manual for the detector will extend filament life.

6.4 FLAME IONIZATION DETECTOR

6.4.1 Introduction

The flame ionization detector (FID) was introduced 1958 by Harley et al. (13) and by McWilliam and Dewar (14). Since then, the FID has become the most commonly used detector for GC. Several factors contribute to the popularity of the FID. First, the FID responds to virtually all organic compounds with favorable sensitivity. The detector response is not affected by modest changes in flow, pressure, or temperature. It does not respond to common carrier gas impurities such as CO_2 and water under normal operation, although trace hydrocarbon levels in the detector gases will affect baseline stability. The linear range extends to about 10^7 orders of magnitude. In the early 1960s, combustion researchers confirmed the reaction that produces ionization in a flame (see Section 6.4.2). Most of the direct evidence was obtained using mass spectrometric techniques to examine the interior regions of hydrogen air flames. Summaries of ionization processes in a flame have been reported by Miller (15) and Bocek and Janak (16).

6.4.2 Operating Principles

The FID consists of a small hydrogen-air diffusion flame burning at the end of a jet, to which the eluted components from the column are directed with carrier gas

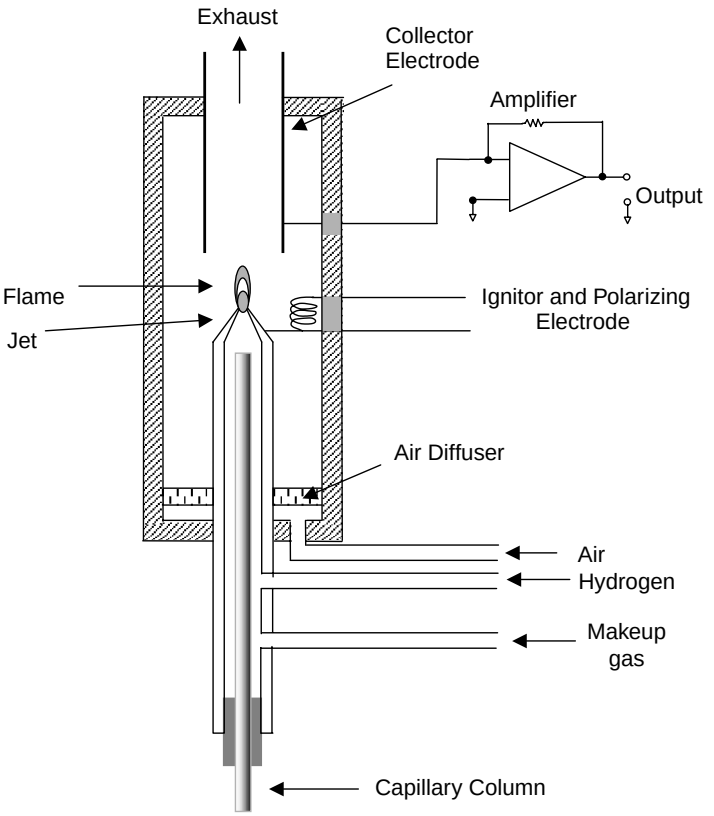

FIGURE 6.12 Schematic diagram of an FID (adapted from Reference 17; published 1971, American Chemical Society).

(and makeup) gas flow. The FID is schematically presented in Figure 6.12. As the organic components reach the flame, electrically charged species are formed. The charged species are collected at an electrode set at a few volts above the flame, producing an increase in current proportional to the amount of carbon in the flame. The resulting current is amplified by an electrometer.

The processes involved in the ionization mechanism in the FID begin at the tip of the jet and occur in discrete regions of the flame (see Figure 6.13). The mixture of carrier gas, makeup gas, and hydrogen flows out of the jet and expands outward. Air flows around the outside of the jet. The heat energy produced at the flame reaction zone preheats the flow of gases from the jet by backdiffusion. The organic materials eluting from the column undergo degradation reactions in this hydrogen-rich region, forming a group of single carbon species. As the two gas flows mix at the reaction zone, with oxygen available, the following reaction occurs:

$$CH^{\bullet} + O^{\bullet} \rightarrow CHO^{+} + e^{-} \tag{6.10}$$

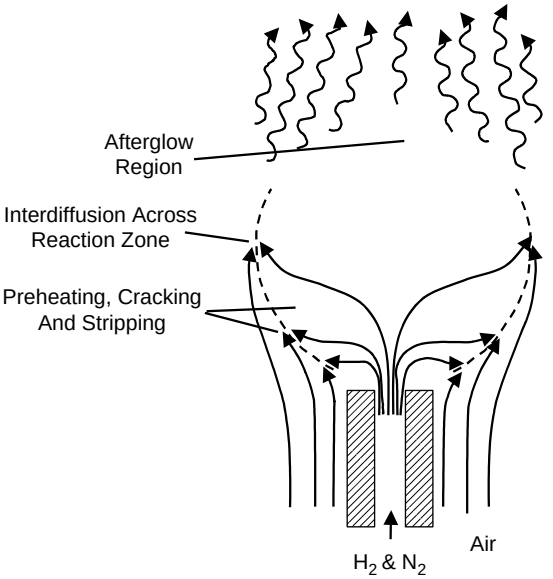

FIGURE 6.13 Schematic diagram of flame process in the FID (adapted with permission from previous edition, copyright 1995 John Wiley and Sons, Inc.)

The CHO^+ species react rapidly with water produced in the flame to generate hydronium ions:

$$CHO^+ + H_2O \rightarrow H_3O^+ + CO \tag{6.11}$$

These positively charge ions and their "polymerized" forms $(H_2O)_nH^+$ are the primary positive charge carrying species. The process occurs approximately once every 100,000 carbon atoms introduced in the flame and it is almost a quantitative counter of carbon atoms being burned. In essence, therefore, the FID response is proportional to the number of carbon atoms, instead of the compound weight or moles.

The process by which the organic compounds produce the single carbon species is not well understood; several schemes, however, have been proposed (18–22). For the flame processes to be effective, there must be a good balance among all the flows and diffusions. Turbulence of the flame must be avoided for proper operation of the detector and baseline stability.

6.4.3 Detector Design

The size of the *jet* tip depends on the type of column and the analysis to be performed. For packed columns, a jet having an internal diameter (i.d.) of 0.018 -in. (about 0.450 mm) is standard, while a jet with a 0.011-in. i.d. is used for capillary columns (about 0.280 mm) for maximum sensitivity (22). The use of the small jet with packed columns may result in flameout and clogging problems. A jet of

0.030 in. i.d. (about 0.760 mm) can be used with 0.53-mm-i.d. capillary columns in such a way that the column is inserted into the jet to within a few millimeters of the flame. This approach has been used to improve peak shape since it prevents the loss of the integrity due to adsorption or possible catalytic decomposition of compounds as they come in contact with hot metal surfaces (23). The fused-silica capillary column, however, should not be allowed to extend beyond that of the jet to avoid noise or spiking, resulting from pyrolysis of the stationary phase and/or decomposition of the polyimide coating on the column.

Diffusion of the gases at the tip of the jet is so central that the detailed shape of the jet affects the detector operation. The tip of the jet should be smooth. In order to avoid blockage, the jet must be kept free from solid support particles from the column, pyrolysis products of the sampling column bleed, and any other contaminants.

The *flowrate of gases* does have an influence on the maximum achievable sensitivity of the FID, as it dictates the particular ratio of carrier gas (or carrier plus makeup gas) to hydrogen (24). The maximum flowrates and the ratio depend on the particular gas used. The FID response is greater with nitrogen as a makeup or carrier gas. The manufacturer instrument manual is the best reference for setting the appropriate gas flowrates. Setting both hydrogen and carrier gas/makeup gas combined at 30 mL/min is a good starting point.

Not all the air enters the reactive zone of the flame; therefore, several times the stoichiometric amount of oxygen is used. For most detectors, typical flows for air are 300–500 mL/min. For uniform and laminar flow along both sides of the jet, many instruments introduce the air through the porous diffusers located below the tip of the jet. Instrument manufacturers clearly specify how to set the air flowrates.

The *exhaust flow* system removes gases and heat out of the detector and in some cases soot and silica caused by large samples, or heavy column loadings, and excessive column bleed. Soot and silica form by incomplete combustion or combustion products in the flame. The use of chlorinated solvents such as chloroform and methylene chloride, for example, will eventually lead to soot formation in the tower of the detector. When occasional soot particles fall back into the flame, it produces spikes in the chromatogram. The silicon-coated column bleed is burned in the flame and combustion products are deposited on the interior of the detector, building an insulating coating. This provides a means for an electrical charge buildup, changing the electric field in the detector. If the detector exit path is cool, water can condense and run back toward the flame, causing electrical shorts. Back diffusion of ambient air into the flame should be prevented. Solvent vapors in the air can be detected by the FID. Air currents near the detector should be avoided; most instruments typically have a mechanism to isolate the flame from air currents.

As is the case with all detectors for gas chromatography, *thermal control* of a FID is an important requirement. The block enclosing the detector should be heated above the temperature of the column to avoid the condensation of sample components in the transfer lines and jet. In addition, the temperature must be

above 100°C to prevent water condensation. Since the temperature of the body of the detector has a slight effect on the detection mechanism it must be stable. However, it is more important to keep the detector from getting too hot since solid surfaces heated by the flame can emit electrons (i.e., thermionic ionization). For example, a large sample or component that can increase the temperature can cause a positive detector response, including samples or components that otherwise would have given a negligible response (e.g., CO). Under such conditions, water may give a negative peak, as it decreases the temperature of the flame. Overheating the detector can also lead to electrical leakage across insulators, causing instability in the detector output current.

The *ion collector* in the FID most typically used is a cylindrical electrode having a large surface to maximize ion collection. The collector is situated above the flame. The ions are driven into the collector by an electric field that is imposed by applying a potential (e.g., several hundred volts) between the collector and a second electrode, typically the burner tip. The generated current is then measured by the electrometer (e.g., a high-impedance operational amplifier). When large amounts of a component are burned, the hydrogen flame becomes more like a hydrocarbon flame, showing an increase in size. In such a situation, the ions are generated higher in the collector, where the electric field is weaker, resulting in a nonlinear response and detector saturation. Near the ion collector, there is typically an ignitor mechanism to light up the flame.

6.4.4 Performance Characteristics

6.4.4.1 Response

The FID is a mass-sensitive detector, responding to the number of carbon atoms entering the detector per unit time. The response factor for the FID is given by the area or peak height divided by the mass of the solute injected (e.g., amperes per gram using peak height). The area response does not change with small changes in carrier-gas flow. The response, however, is affected by the presence of heteroatoms like O, S, and halogens. A list of compounds with little or no response in the FID is provided in Table 6.6.

To estimate relative response for any compound, the effective carbon number (ECN) was introduced. This number accounts for the fact that the FID response decreases in the presence of certain heteroatoms. To calculate the ECN, a value

TABLE 6.6 Compounds with Little or No Response in the FID

He	N_2	H_2S	NO	CCl
Ar	O_2	CS_2	N_2O	$SiCl_4$
Kr	CO	COS	NO_2	CH_3SiCl_3
Ne	CO_2	SO_2	N_2O_3	SiF_4
Xe	H_2O	SO_3	NH_3	$SiHCl_3$
		HCN		

is assigned to particular groups of atoms, relative to the response of a reference material, for which the ECN is simply the number of carbons in the molecule, usually an *n*-paraffin. The ECN parameter is then obtained by adding the contributions from all the atoms or functional groups in the molecule. A set of values used to calculate the ECN is given in Table 6.7 (18,25,26). The ECN for butyric acid, for example, would be 3.0, having three aliphatic carbons (1.0 each) and one carboxylic group (0.0). Similarly, the ECN for ethanol would be 1.4. A large number of experimental relative response factors have been tabulated (27–30). A good use of the ECN is to determine the relative response factor of compounds for which standards with sufficient purity are not available. A discrepancy between theoretical and experimental response factors are indicative of adsorption or decomposition of solutes in the chromatographic system, particularly when the usual signs of poor peak shape and tailing are not observed. One must be aware, however, that response factors can be detrimentally diminished by improperly set flow parameters, high column bleeding, and water in the air supply.

6.4.4.2 Noise, Detection Limits, and Linearity

The FID is most noted for its linear range ($\sim 10^7$). It also exhibits LOD as low as 10^{-13}g·C/s and low noise. Under normal conditions, background currents of 10^{-13} Amperes or less can be obtained. In general, the FID is rugged, easy to operate, and the most popular detector in gas chromatography. A disadvantage of the FID is that it destroys the sample.

6.4.5 FID Modifications

The FID can respond to inorganic gases by operating in a hydrogen-rich mode and mixing with oxygen, instead of air, to support combustion. The commercial

TABLE 6.7 Contributions to Effective Carbon Number by Different Atom/Groups

Atom	Type	Contribution to ECN
C	Aliphatic	1.0
C	Aromatic	1.0
C	Olefinic	0.95
C	Acetylenic	1.30
C	Carbonyl	0.0
C	Nitrile	0.3
O	Ether	−1.0
O	Primary alcohol	−0.6
O	Secondary alcohol	−0.75
O	Tertiary alcohol, esters	−0.25
Cl	Two or more on single aliphatic C	−0.12 each
Cl	On olefinic C	0.05
N	In amines	Similar to O in corresponding alcohols

detectors are easily modified by introducing the oxygen with the carrier gas while hydrogen is introduced into the detector through the inlet provided for air. In such an arrangement, the detector is known as the hydrogen atmosphere flame ionization detector (HAFID) LOD reported for some gases when operating in the HAFID mode are shown in Table 6.8 (31). An enhanced detection of organometallic compounds containing iron, tin, lead, molybdenum, and tungsten is also achieved by doping the hydrogen flow with hydrides (32). The HAFID response for silicon containing compounds is also enhanced by doping the flame with ferrocene, to produce a silicon-to-carbon selectivity of 10^4 (33).

Another modification of the FID is the electrolyzer-powered FID (EFID) (34). In this adaptation, the FID is slightly modified to operate with a combustible, stoichiometric, premixed gas mixture of hydrogen and oxygen. The two predominant modifications to the FID are (1) a flame tip with a hole of about 0.25 mm to prevent flashback and (2) that the detector must be maintained above 100°C to prevent water condensation. The gas mixture is produced by means of water electrolysis, without gas separation or compression, at a low flowrate, and does not need He makeup gas. Water electrolysis generated by a low power electrolyzer can provide the total gas consumed for prolonged periods of time before water replenishing is required, which typically allows days of continuous operation. For example, a small electrolyzer requiring 3.6 W (1.5 A at 2.4 V), can consume about 12 mL of water per day. Care must be taken to use clean triple distilled water to minimize the flame background current.

The EFID provides sensitivity similar to that of the conventional FID with linearity of six orders of magnitude but with an improved detectability (at least twice). It is selective to carbon containing compounds, with a response of about 30 percent lower for aliphatic compounds than for aromatic compounds and no observable difference for nitrogen-, sulfur-, phosphorus-, and chlorine-containing compounds. The obvious advantage of the EFID is the elimination of gas cylinders and regulators required for a conventional FID. This reduces costs associated with operation. With the elimination of cylinders the portability of the gas

TABLE 6.8 LOD for Some Gases for the HAFID

Gas	LOD (g/sec)
CH_4	2×10^{-11}
CO_2	4×10^{-8}
H_2S	4×10^{-10}
NO	2×10^{-11}
O_2	5×10^{-8}
SO_2	4×10^{-10}
N_2O	7×10^{-9}
NO_2	2×10^{-9}
CO	4×10^{-7}
He	5×10^{-8}

chromatograph becomes attractive. In fact, it has been demonstrated that the EFID can be used for gas-cylinder-free GC, suitable for fieldwork (35). In such an approach, the electrolyzer produces an oxygen–hydrogen gas mixture that can be used for sample desorption and sweeping in a purge-and-trap sampling system, as the analytical column carrier gas, and as the combustible gas mixture.

6.4.6 Other Practical Considerations

1. Incorrect adjustment of the flame can result in problems with the FID. It is important to adjust the flame size and ratio of its gases for proper operation. After changing the jet, carrier-gas flow or sample size, one should consider the effect that these can cause to the flame and make adjustments, if necessary.
2. Contamination is another source of problems with the FID. Sources of contamination include impure gases, short-circuiting out of the detector by water, and deposits in the detector tower around the jet (e.g., soot and silica).
3. One must use the proper-size jet and nitrogen as the makeup gas for maximum sensitivity.

6.5 ELECTRON-CAPTURE DETECTOR

6.5.1 Introduction

The electron capture detector (ECD) is one of the most popular and valuable gas chromatographic detectors in use today, perhaps second after the FID. The ECD makes use of a β emitter radioactive source to produce electrons on collision with the carrier gas, producing a standing current that is measured. The presence of a particular species eluting from the chromatographic column can capture electrons and a decrease in the standing current is registered. This detection method, based on gas-phase electron-capture reactions, can respond to picogram and even femptogram levels of specific substances in complex matrices. This ability makes it a very useful detector for environmental and biomedical studies.

Many developments led to the introduction of the electron-capture detector in 1960 (36). The first was the invention of the β-ray ionization cross-sectional detector in 1951. This detector was modified by Lovelock in 1958 to produce the β-ray argon detector (37). The Lovelock variation placed a 1000 V potential across the electrodes of the β-ray ionization cross-sectional detector and substituted hydrogen gas with argon as the carrier gas. In such a configuration, argon absorbs the β-radiation from the radioactive source, initiating ionization. Because of the potential applied between the two electrodes, the electrons produced are accelerated toward the anode. At high potentials, electrons develop sufficient kinetic energy to produce excited metastable argon species on collision with argon atoms. These argon species have enough energy (i.e., 11 eV) to ionize most organic molecules. Anomalies were observed when halogenated

compounds were eluted, so Lovelock proposed the theory that electronegative species, functionally present in an organic molecule, could capture an electron to form a negatively charged species:

$$CX + e^- \rightarrow CX^- + \text{energy} \tag{6.12}$$

These entities would then cause a reduction in the standing or background current. This phenomenon, known as *electron capture*, is observed more readily at lower electrode potentials.

6.5.2 Operating Principles and Variables

6.5.2.1 Cell Design and Radiation Source

Cell Design The design of the electron capture detector is a simple arrangement of a chamber containing two electrodes with a source of radiation to induce ionization. Figure 6.14 shows two different cell geometries. Early cells with coaxial geometry were usually converted argon ionization detectors, where the anode is found along an axis sheathed by the cathode containing the radioactive source. The pincup detector, as illustrated in Figure 6.14a, can be viewed as a modified chamber of a coaxial ECD geometry. Another geometry, the concentric cylinder ECD, has the radioactive foil located in the cathode region and the anode is in an isolated region. Another design, the *plane parallel electron capture detector*,

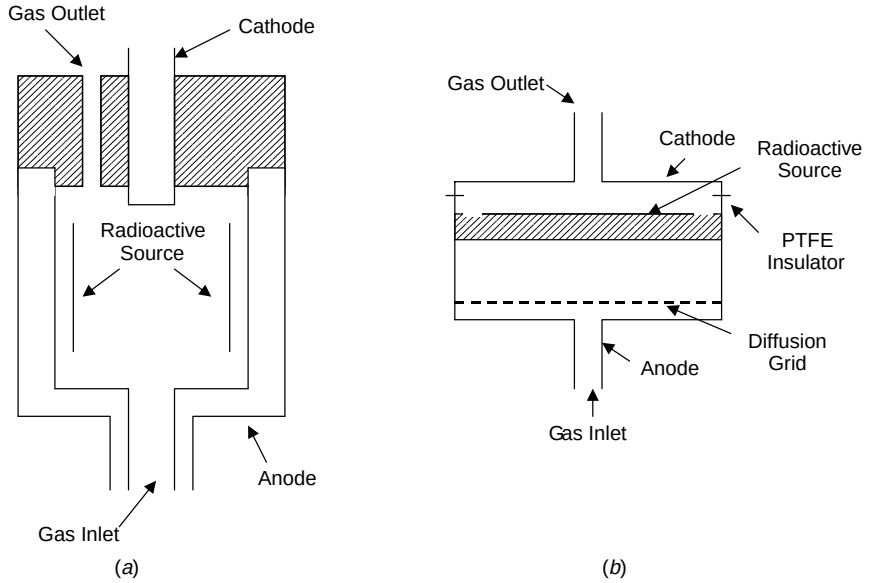

FIGURE 6.14 ECD cell designs: (a) modified chamber of a coaxial ECD geometry (pincup detector) and (b) plane-parallel electron-capture detector (adapted from Reference 44 with permission, copyright 1974 Elsevier).

is illustrated in Figure 6.14b. The anode and cathode in this design are parallel and the flow of the carrier gas is in a direction opposite to the motion of the negatively charged species. This design is considered more efficient. A cell design also exists to promote gas-phase coulometry (38). Electron-capture coulometry occurs when the ratio of electrons captured per second (coulombs) to the number of molecules through the detector in a second approaches one. Coulometric detection provides the best sensitivity, but has limited linearity.

Radiation Source The most common form of the ECD uses ^{63}Ni as the radiation source, although tritium, which is a weak β emitter, is also commercially available. The β-rays from tritium have a range of only 2.0 mm, thus requiring a short cell distance for efficient currents. The range of ^{63}Ni is much larger at 8.0 mm. The maximum operating temperatures for tritium and ^{63}Ni are 225 and 400°C, respectively. The lower limit is determined by the column conditions. If the temperature is too low, condensation of high-boiling-point eluents and stationary phase bleed may occur. The ionization chamber must be well insulated to keep the detector temperature stable to better than $+/-0.1$°C because the number of electrons emitted from the source, their energies and the electron capture mechanism are all temperature-dependent. One advantage of tritium is that the flux of radiation is higher, so ionization is more efficient. However, tritium is easily contaminated by adsorbed components on the foil that shields the weak (18-keV) β-rays. The upper temperature limit of a tritium ECD is approximately 220°C. At higher temperatures, tritium emanates with the effluent from the detector at a level that constitutes a health hazard according to the Nuclear Regulatory Commission (NRC), and should therefore be vented into a fume hood. These problems and inconveniences are minimized with the ^{63}Ni foil. If it is overheated, no radioactive material escapes the detector; instead, a loss of activity occurs by diffusion of the ^{63}Ni into the foil. The ^{63}Ni detector can usually be cleaned by periodically operating the detector at a high temperature. Alternatively, the ^{63}Ni source can be purchased as a "sealed source" for which the vendor holds the necessary radiological license and the user is required only to perform relatively simple radiological tests.

6.5.2.2 Flowrate

There are many requirements for flowrate and gas composition. The ECD requires a gas that can efficiently attenuate the beta radiation to create a population of positive ions and electrons in the detector, and rapidly thermalize these secondary electrons. As the concentration of free electrons increase, the probability of electron capture also increases. Optimum performance is seen from gases with large ionization cross sections, such as nitrogen or argon containing 5–10% methane. Gases such as helium and hydrogen with low ionization cross sections will not work. If hydrogen or helium is used, the flowrate must be kept below 5 mL/min with nitrogen or methane/argon as the makeup gas at a flowrate of 20 mL/min or higher. Higher flowrates of hydrogen greatly affect the linearity and sensitivity. If the source is tritium, hydrogen use decreases its activity and lifetime. Constant

flow control should be used at all times to minimize baseline and response instability. If a pressure regulator is used, a makeup gas should be used to minimize the reduction of carrier-gas flow within a column of increased temperature.

A dopant in a carrier gas can enhance the response of certain compounds. In such a case, a makeup gas is doped with a particular reagent, such as traces of oxygen or nitrous oxide, which produce ion–molecule reactions inside the detector chamber (39–42). The detector response signal is dictated by the kinetic and thermodynamic characteristics of the ion–molecule reaction. Albeit a small sacrifice in electron population, the addition of parts per thousands of O_2 in the carrier gas can increase the signal response for hydrocarbons and hydrogenated hydrocarbons significantly, following the following scheme:

$$e^- + O_2 \rightleftharpoons {O_2}^-$$
$${O_2}^- + M \rightleftharpoons \text{products of } M + e^-$$

A 400-fold increase in response has been reported for weak electrophores, such as benzo[*e*]pyrene (38). In the case of N_2O, the following mechanism has been proposed:

$$e^- + NO_2 \rightarrow O^- + N_2$$
$$O^- + N_2O \rightarrow NO^- + NO$$
$$NO^- + M \rightarrow \text{products of } M + NO + e^-$$

Using such a dopant, the response for vinyl chloride (41), CO_2, H_2, and CH_4 has been enhanced (42).

6.5.2.3 Voltage

Potential is applied to the electrodes of the ECD in several ways: constant voltage, pulsed constant frequency, and pulsed variable-frequency constant current. The magnitude of the applied potential in the DC mode is a critical parameter dependent on the species measured, cell design, carrier-gas composition, and detector contamination. Competing processes can also occur under DC conditions (43), resulting in anomalous behavior (see Figure 6.15). Therefore, pulsed voltage is preferred to overcome this behavior. Pulsed voltage significantly decreases the buildup of charged zones in the detector resulting from differences in the velocity of positively charged ions compared to the mobility of free electrons. The pulse can be visualized as a process for collecting electrons in Figure 6.16. Electrons having a thermal energy will attach to any electron-capturing molecule when no pulse is applied, and negatively charged ions are produced. The negative ions recombine with positively charged ones and a reduction in the standing current is observed. Only the fast moving electrons are collected since the period of the pulse is adjusted so the slow-moving negatively charged species are not collected. The operating variables are pulse amplitude, pulse frequency, and pulse duration. Commonly, the pulse reaches 30–50 V over a time interval and is repeated every

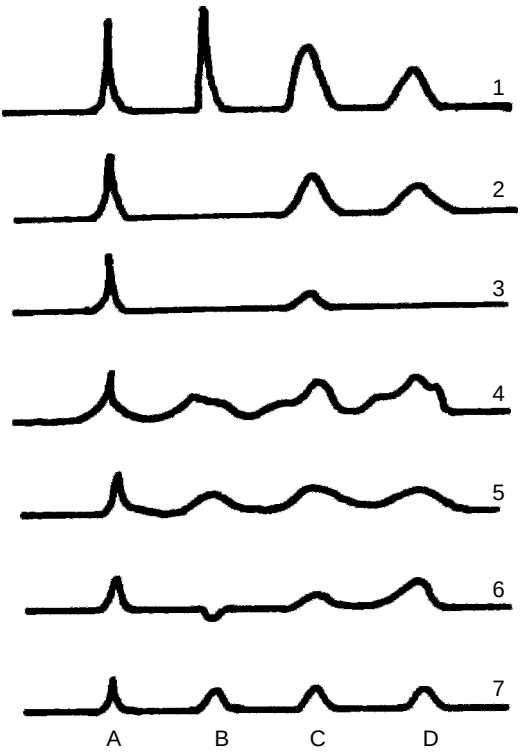

FIGURE 6.15 Anomalous responses that can be seen in a ECD operating in the DC mode: (1) chromatogram that truly represents a sample mixture, where *A* and *D* are peaks for electron absorbers, *B* is a large amount of a nonabsorber, and *C* contains unresolved absorbing and nonabsorbing components; (2) ECD operating correctly; (3) ECD losing peaks as a result of space charge effects; (4) ECD with contact potential (result of material adsorbed on electrode) enhancing the applied potential; (5) contact potential opposing the applied voltage (observe increased tailing and false peak at *B*); (6) ECD acting as if it were an argon detector (note inversion of *B* and reduction of peak *C*); (7) ECD operating as an electron mobility device and an ECD (note the false peak at *B*). (Reprinted with permission from previous edition. Copyright 1995 John Wiley and Sons, Inc.)

100 μs. The pulse itself can be as short as 0.1 μs if the argon/methane mixture is used, but is at least 1.0 μsec in duration if nitrogen is used. A significant difference between DC mode and pulsed mode is that the driving force of the applied potential field is absent and the electrons attain thermal equilibrium. Enhanced detectabilities are seen in this pulsed mode.

Pulsed variable frequency with constant current mode is considered the superior method and is used by most manufacturers of ECDs. A preselected level of current is required. When an electron-capturing solute enters the detector, the standing current decreases; the electronic circuit is adjusted to a frequency of pulsing to maintain a constant current. The detector response is in the frequency

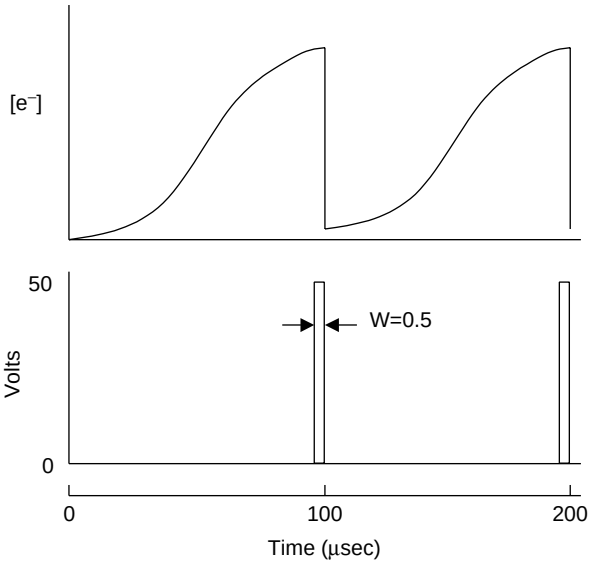

FIGURE 6.16 ECD pulse mode (adapted from Reference 44 with permission, copyright 1974 Elsevier).

change instead of a decrease in current as in the other modes. The dynamic range and sensitivity are improved in the constant current mode.

6.5.3 Performance Characteristics

6.5.3.1 Response

In the ECD, the reaction of electrons with electron-absorbing compounds is a second-order rate mechanism that can be affected by temperature as shown in Figure 6.17. It is also affected by the electronegativity of the species itself, the presence of other species, and the energy of the electrons. Even when these conditions are controlled, variations have been observed. To minimize this, suitable operating parameters and strong electron-absorbing molecules are used to force the forward rate of reaction to approach a pseudo-first-order rate for the observed coulometry. A response factor could be calculated if the kinetics of the reaction were more fully understood.

A convenient approach for pulsed ECD was proposed by Sullivan (45). It was shown that the output frequency F and solute concentration in the detector cell [A] are related by

$$F = \frac{1}{K(k_1[\mathrm{A}] + K_\mathrm{d})} \tag{6.13}$$

where k_1 is the rate constant for electron capture, K_d is the pseudo-first-order rate for reassociation of electrons with positive ions and is generally small, and K is

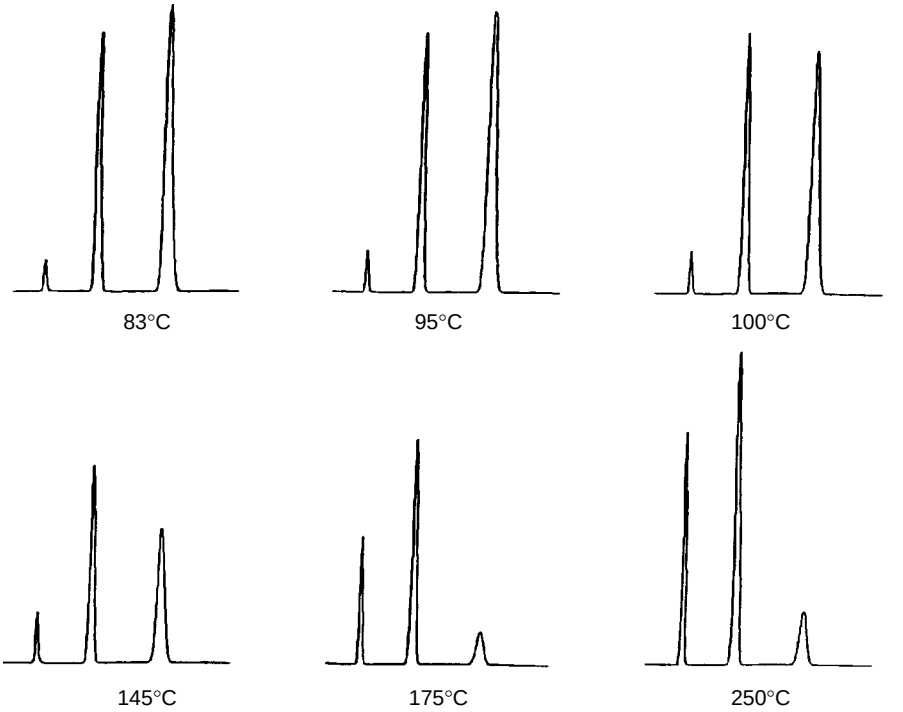

FIGURE 6.17 Influence of detector temperature on ECD response (reprinted with permission from previous edition, copyright 1995 John Wiley and Sons, Inc.)

a constant of proportionality. The output current I can be expressed as

$$I = \frac{I_s}{K(1 - e^{-k})} \tag{6.14}$$

where I_s is current produced by the radioactive source, $K = (k_l[A] + K_d)t_p$, and t_p is $1/F$. Substitution of values for I and I_s in the equation and the use of reiterative techniques yields an approximate value of K, so that

$$k_l = \frac{KfM \times 10^{12} Z}{Nr} \tag{6.15}$$

Here, f is flow (mL/s), N is Avogadro's number, r is a frequency-to-voltage correction factor for analog output (μV/Hz), M is the analyte molecular weight, and Z is the area response factor (μV·s/pg).

Wentworth and Chen developed a kinetic model using a parallel-plate tritium source for the electron-capture detector that correlates observed response values with electron affinities derived from reversible half-wave potentials in aprotic

solvents and temperature dependence of the kinetics and the thermodynamic properties of the solute (46,47).

The response factor of the ECD will vary with the nature of the solute since it is a selective element detector; generalizations, however, have been made (48). Table 6.9, for example, lists relative responses of various classes of compounds. For quantitation, however, internal and external standards are recommended.

TABLE 6.9 Relative Response Values Using ECD (K' Based on Chlorobenzene = 1)

Chemical Classes	K'	Selected Samples
	0.01	
Alkanes, alkenes, alkynes, aliphatic ethers, esters, and dienes		Hexane Benzene Cholesterol Benzyl alcohol Naphthalene
	0.10	
Aliphatic alcohols; ketones; aldehydes; amines; nitriles; monofluoro and monochloro compounds		Vinyl chloride Ethyl acetoacetate Chlorbenzene
	1.0	
Enols; oxalate esters; monobromo, dichloro, and hexafluoro compounds		*cis*-Stilbene *trans*-Stilbene Azobenzene Acetophenone
	10.0	
Trichloro compounds, chlorohydrates, acyl chlorides, anhydrides, barbiturates, thalidomide, and alkyl leads		Allyl chloride Benzaldehyde Tetraethyl lead Benzyl chloride Azulene
	300	
Monoiodo, dibromo, trichloro, and mononitro compounds; lacrimators; fungicides; and pesticides		Cinnamaldehyde Nitrobenzene Carbon difulfide 1,4-Androstadiene-3,11,7-trien Chloroform
	1,000	
1,2-Diketones; fumarate esters; pyruvate esters; quinines; diode; tribromo, polychloro, and dinitro compounds; and organomercurials		Dinitrobenzenze Diiodobenzene Dimethyl fumarate Carbon tetrachloride
	10,000	

Source: Data obtained from Reference 48.

6.5.3.2 Linear Range and Detection Limits

The response of the ECD is linear with concentration over only two orders of magnitude in the DC mode. A relationship was proposed by Wentworth et al. (49) with a linear range of about four orders of magnitude as follows:

$$\frac{I_s - I}{I} = kc \tag{6.16}$$

In Equation 6.16, I_s is the standing current, I the current measured when electron-absorbing species are in the detector at concentration c, and k is a constant characteristic to the cell and the species present (also known as the *electron-capture coefficient*).

To increase the linear dynamic range of the detector response (i.e., linearization), it can be operated in the pulse mode by varying the pulse interval to maintain a constant current. This method of constant current—variable frequency is generally used in commercial instruments for linearization. Generally, manufacturers report a linear dynamic range of about 10^4 and LOD of 5–100 fg/s for lindane (50). The linear dynamic range can be extended by using analog converters and several manufacturers have developed microprocessor-based linearizers for use in constant frequency instruments.

6.5.4 Nonradioactive ECD: Pulse Discharge ECD

There have been several attempts to develop a nonradioactive electron-capture detector for GC. The first nonradioactive detector was introduced in 1964, which was commercialized for a brief period of time (51). Electrons were generated by an electrical discharge in a carrier gas prior to mixing with the gas chromatographic column effluent. The detector seemed to require critical flowrate control and this created serious problems. Other nonradioactive methods have been reported for the formation of electrons (52–55), but none have become commercially available. These include the use of a hydrogen Lyman alpha emission to ionize a dopant gas in argon (52), the use of a thermionic emitter as the source of electrons (53), ionization of a dopant gas using a resonance lamp source with MgF_2 window (54), the use the photoelectric effect with UV radiation on a metal (55), and using a microwave discharge to produce metastable helium species (56).

A commercially available nonradioactive ECD is the pulse discharge detector, which uses a stable, low-powered, pulsed DC discharge in helium as the ionization source; the detector can also be configured as a helium ionization or photoionization detector (see Section 6.8.2.2). The commercial version of the detector seems to have been born out of the work by Wentworth and co-workers, which started with the use of the microwave induced discharge in helium as the ionization source (56), leading to the pulse discharge detector (57). The pulse discharge electron-capture detector (PDECD) configuration is schematically shown in Figure 6.18. The solutes eluting from the column enter the detector chamber in the opposite direction of the flow of helium from the discharge zone and are

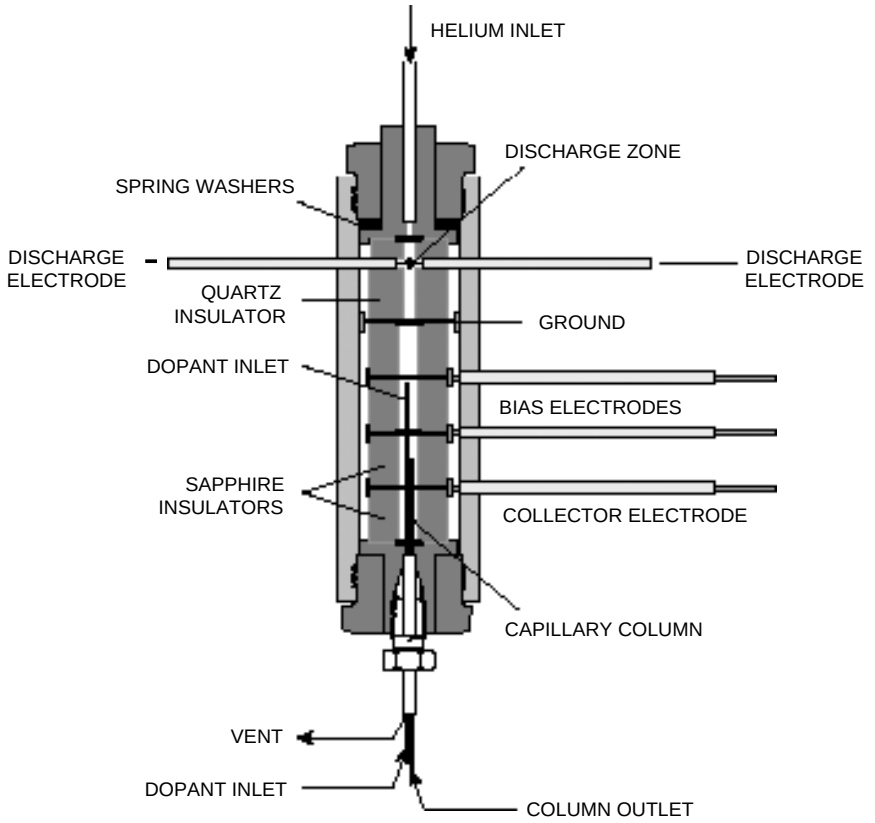

FIGURE 6.18 Schematic of the PDECD (illustration courtesy of VICI Valco Instruments Co. Inc.)

ionized by photons from the helium discharge above the column end. The main source of ionization is the radiation from the diatomic helium He_2 ($A^1\Sigma_u^+$) excited state to the dissociative 2 He ($1S^1$) ground state; the photon energy from the He_2 continuum is in the range of 13.5–17.7 eV. The electrons produced during the ionization process are directed toward a collector electrode by two bias electrodes and the current monitored by an appropriate amplifier. The PDECD requires the addition of a gas dopant for generation of thermal electrons (3% xenon in helium is recommended by manufacturer, although CH_4 can also be used). First, the dopant gas is ionized by the photons from the discharge and the resulting electrons produce the detector standing current. The presence of an electron-capturing compound in the detector results in a decrease in the detector standing current; this decrease in current constitutes the PDECD response. It is important to indicate that the purity of helium must be 99.999% or better and must be used in conjunction with a gas purifier that is included as part of the commercial unit. More details on the detector operation are given in Section 6.8.2.2.

As the conventional ECD, the PDECD is selective to compounds with high electron affinity (e.g., chlorinated pesticides). The LOD is in the femtogram level, with response characteristics and sensitivity similar to those of the ECD using a ^{63}Ni source. The signal can also be described by Equation 6.16. For linearization, the detector utilizes a feedback system to the bias electrode. The detector output current is compared with a reference value and in the presence of an electron-capturing solute; the bias voltage is raised to maintain a constant current. Such voltage is the linearized output signal corresponding to the concentration of the electron-capturing solute, resulting in a linear dynamic range of five orders of magnitude.

6.5.5 Other Practical Considerations

1. Oxygen, which is an electron absorber, should be scrupulously trapped by molecular sieves.
2. All tubing and septa should be cleaned and baked out in a vacuum oven before installation.
3. Column bleed should be kept to a minimum.
4. Stationary phases with high electron affinity such as trifluoropropylmethyl silicones (OV-202, OV-210, OV-215, etc.) should be avoided.
5. The system must be leaktight to avoid diffusion of gases.
6. When dealing with radioactive isotopes one must exercise the necessary precautions.

6.6 THERMIONIC DETECTOR

6.6.1 Introduction

The thermionic detector (TID), also known as the nitrogen–phosphorous detector (NPD), is based on the phenomenon that a metal anode emits positive ions when heated in a gas. It is a commonly used gas chromatographic detector for the selective determination of organic compounds containing nitrogen (N) and phosphorus (P) atoms. These include the detection of pharmaceuticals, pesticides, and environmental pollutants. The detector would appear to function as the FID; however, its operation is based on a completely different principle.

The TID evolved from the earlier alkali flame ionization detector (AFID) with sodium salt deposited on a metallic probe inserted in the flame (see Figure 6.19). This detector showed specificity for phosphorous and halogen-containing molecules, but the selectivity was poor. Karmen (58) obtained a phosphorous–hydrocarbon selectivity of 105:1 by stacking two flames. The first flame had the purpose of burning eluted materials from the column and vaporizing sodium that had been deposited on a platinum screen located above the flame. The vapor was then transferred into the second flame where ionization took place. Other design modifications exist, but designs with the salt around the burner were

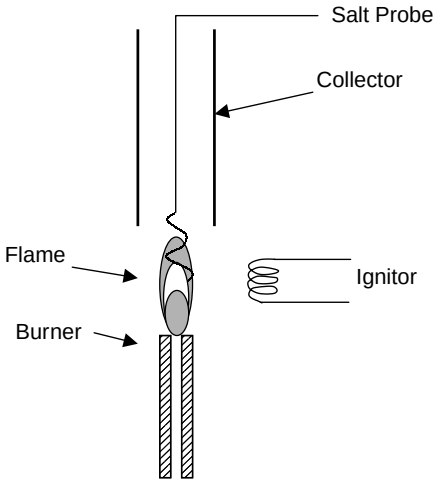

FIGURE 6.19 Schematic of the probe in the flame of the alkali flame ionization detector.

preferred, as the salt in these designs was not diminished as quickly, equilibrium was established more rapidly and sensitivity was improved. The AFID, however, was difficult to use because frequent adjustments of independent parameters were required, the metal salt degraded quickly, the background signal was unstable, and the sample responses were not reproducible.

In 1974, Kolb and Bischoff designed and developed what is the modern thermionic detector, specific for nitrogen and phosphorous compounds (59). The new detector had three significant features that are the base for the modern instruments: (1) a glass bead containing nonvolatile rubidium silicate was used instead of a pellet of volatile alkali metal salt, but modern instruments make use of rubidium or cesium silicate beads; (2) the bead was fused onto a platinum wire providing a means for electrically heating the bead instead of using a flame; (3) the hydrogen flow necessary for nitrogen and phosphorus response was only a few milliliters per minute, so there was no flame. The detector design is illustrated in Figure 6.20. Compared to the AFID, this new thermionic detector exhibited longer life of the alkali-impregnated component, better baseline stability, more reproducible response, and better control of key operating parameters.

6.6.2 Operating Principles and Variables

6.6.2.1 Mechanism

The system involved in the thermionic detector is complex and one may argue that a comprehensive theory underlying its principle of operation does not exist. Originally, the TID was viewed as a modification of the AFID, as a gas-phase ionization process occurring in the layer immediately adjacent to the hot thermionic source (59,60). Later, however, it was recognized that intricate surface phenomena

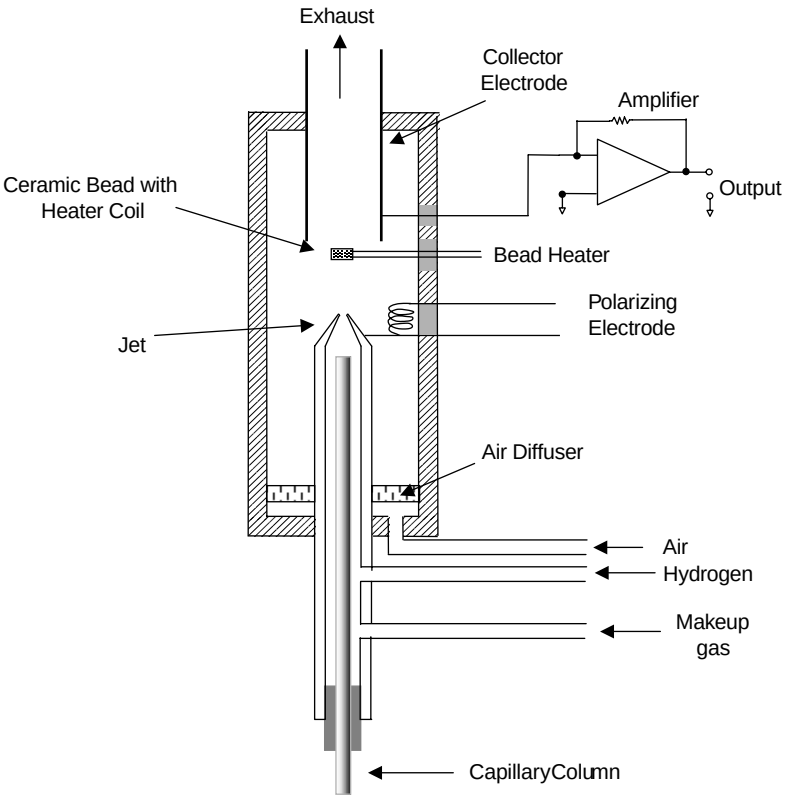

FIGURE 6.20 Schematic drawing of the NPD (adapted from Reference 17, published 1971 American Chemical Society).

are operating, hence, leading to a surface ionization process occurring on the hot surface of the thermionic source (61). On entering the detector, sample compounds are decomposed in the hot, chemically active surface layer and the decomposition products are ionized. The gas environment of the detector is a dilute mixture of hydrogen in air. The detector does not begin to function without the highly reactive chemical environment from a series of chain reactions that begin when there is enough thermal energy to dissociate H_2 molecules into reactive H atoms. Unlike the FID, there is no self-sustaining flame and the chemistry exists only near the hot thermionic source surface. The thermal energy is provided by heat, which is accomplished by applying current to a coil containing the thermionic source. When the heating current is turned off, the layer at the surface of the source ceases to exist and the response disappears.

The realization of surface chemistry at the thermionic surface led to better understanding of key parameters such as the electronic workfunction of the thermionic surface, which is determined by the chemical composition of the surface, the temperature of the thermionic surface, and the composition of the gases

close to the thermionic surface. Adjusting these key parameters has made it possible to operate the TID in different modes beyond the nitrogen–phosphorus mode (62). The use of the TID in the nitrogen–phosphorus mode, however, is the most popular one.

6.6.2.2 Flowrate and Heating Current

The detectability and the specificity of the detector are both affected by the magnitude of the hydrogen flow to the detector and the magnitude of the heating current. The hydrogen flow affects the concentration of the hydrogen atoms in the reactive gaseous layer around the thermionic source that in turn determines the response. The effect of the flowrate is illustrated in Figure 6.21. Increasing the source heating current beyond the base value required to initiate the H_2/air chemistry can increase the detectability of the detector by a factor of 10. The temperature of the source is affected by the heating current, the auxiliary heating of the detector walls, the thermal conductivity of the gas mixture flowing past it, and the volume of the gas flowing past it. Changes in any of these parameters are important since the source must remain hot enough to produce a reactive chemical environment. The temperature gradient between the source and the walls should be minimized by operating the auxiliary heater for the walls at a sufficiently high setting. This also minimizes the effect of large concentrations of sample passing through the detector. If helium is used as a makeup gas instead of nitrogen, a higher heating source current is required due to its much higher thermal conductivity.

The hydrogen and makeup gases mix with the column effluent inside the jet. The gases leave the jet and mix with air; then, they are heated at the hot thermionic source, where decomposition and ionization occur. Ions are then collected at the collector electrode, which is maintained at a potential of a few hundred volts. Typical gas flows are 3–4 mL/min hydrogen, 100–200 mL/min air or

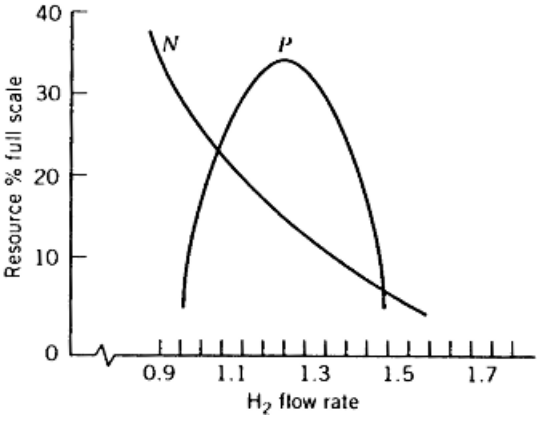

FIGURE 6.21 Effect of flowrate on selectivity for the TID in the NPD mode (reprinted with permission from previous edition, copyright 1995 John Wiley and Sons, Inc.)

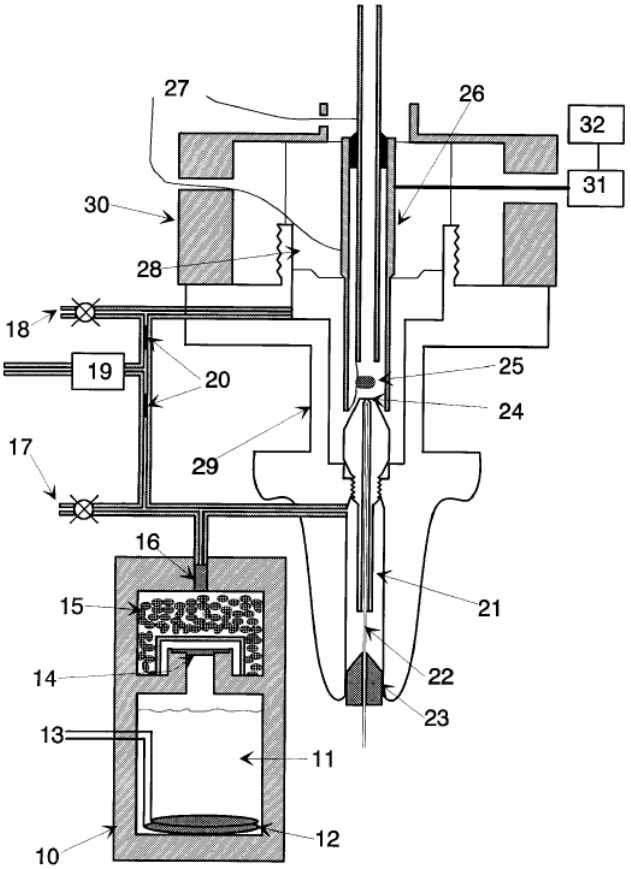

FIGURE 6.22 Illustration of the ENPD. The water electrolyzer consists of a container (10) filled with an aqueous solution of KOH (typically 0.3 M) (11) and two nickel-mesh electrodes (12) connected to an external power supply through electrical feedthroughs (13). The generated H_2/O_2 gas mixture passes through a 10–20-μm Teflon membrane (14) for partial separation from the water mist and is further dried by the silicagel (15). The gas mixture then passes through a frit-type flow restrictor element (16) and flows to the ENPD. Helium makeup gas (17) is mixed with the electrolyzer output, and air is supplied around the jet (18). Alternatively, the valves of the helium and air are shut off, and both makeup gas and surrounding air are supplied by a miniature air pump (19), with two flow restrictors (20) to split the airflow. The analytes are introduced into the jet (21) through the GC effluent from a column (22) sealed by a ferrule (23). The analytes are swept by the makeup gas and the electrolyzer output through the narrow aperture (24) toward the active source (25). The active source is attached to the collector assembly (26), heated by a high-frequency AC current (27), and the combination of high temperature and H_2/air mixture forms the active atmosphere needed for the analyte decomposition. The collector assembly is held by a Teflon insulator (28), which also serves as a gas seal. The detector is mounted on the FID-type detector base (29) and covered by a clamp (30). The ions formed on the active source are collected by the biased collector assembly (26), and the current is amplified by an amplifier (31) and recorded by an integrator (32). (Reprinted from Reference 63 with permission. Copyright 1997 John Wiley and Sons, Inc.)

oxygen, and 30–40 mL/min makeup gas (typically He). A relatively new design incorporates the features of the electrolyzer-powered FID (34) (see Section 6.4.5) to produce the necessary gases to operate the TID in the NP mode, which has been named the electrolyzer-powered nitrogen–phosphorus detector (ENPD) (63). The ENPD is illustrated in Figure 6.22.

The TID response deteriorates with time, which is the major disadvantage of this detector. This is due to the loss of rubidium or cesium during the operation of the detector. Therefore, regular replacement of the bead containing the thermionic source is necessary for detectors in continuous use. An alternative to the thermionic bead has been proposed in which the gas chromatograph is operated in the AFID mode and the alkali is introduced via an aerosol into the FID (64). This approach, however, requires several modifications and additions to the gas chromatograph, which may be inconvenient for routine operation.

6.6.3 Performance Characteristics

Response factors in the thermionic detector are directly related to experimental conditions and vary for the nitrogen and phosphorous modes as well as the nature of the compound containing the heteroatom. Selectivities typical of the ratio of nitrogen to carbon range from 10^3 to 10^5 gN/gC. For phosphorous to carbon the range is 10^4 to 5×10^5 gP/gC. The linear dynamic range varies from 10^3 to 10^5 and differs for each compound examined. Using the appropriate column and detector parameters, traces of herbicides of 0.5 ng, for example, can be easily determined.

6.6.4 Other Considerations

1. Stationary liquid phases that contain nitrogen or phosphorous, such as OV-225, OV-275, FFAP, XE-60, TCEP [1,2,3-tris (2-cyanoethoxy)propane], and TCEPE (tertracyanoethylated pentaerythritol) should be avoided.
2. Halogenated solvents should be avoided to prevent their decomposition and their alteration of ionization characteristics that may occur.
3. Silylation reagents should be avoided since they leave deposits on the thermionic surface and cause a loss of response.
4. If halogenated solvents or silylation reagents cannot be avoided, their effects can be minimized by turning off the bead current while the solvent is eluting; however, it should not be off longer than 2 min.

6.7 PHOTOIONIZATION DETECTOR

6.7.1 Introduction

The photoionization detector (PID) uses UV radiation as a means of ionizing the analyte exiting the chromatographic column. It was introduced by Lovelock

in 1960 (65), with a design that used an argon glow discharge as the source of UV radiation for ionization. The detector was not popular since it demanded operation in a vacuum, was unstable, was easily fouled by column bleed, and required a skilled operator. Almost 15 years later, the PID reappeared in a design that separated the energy source and the ionization chamber, giving an improved stability (66). After further modifications (67), the PID also gave a large linear range (i.e., 10^7) and lower background and reduced the appearance of column bleed. These improvements resulted in the commercial introduction of the PID. Modern detectors make use of a lamp as the source of UV radiation; an illustration of a PID is shown in Figure 6.23.

6.7.2 Operating Principles

When a molecule absorbs a photon of light of sufficient energy, the molecule can dissociates into its parent molecular ion and an electron; such a photoionization

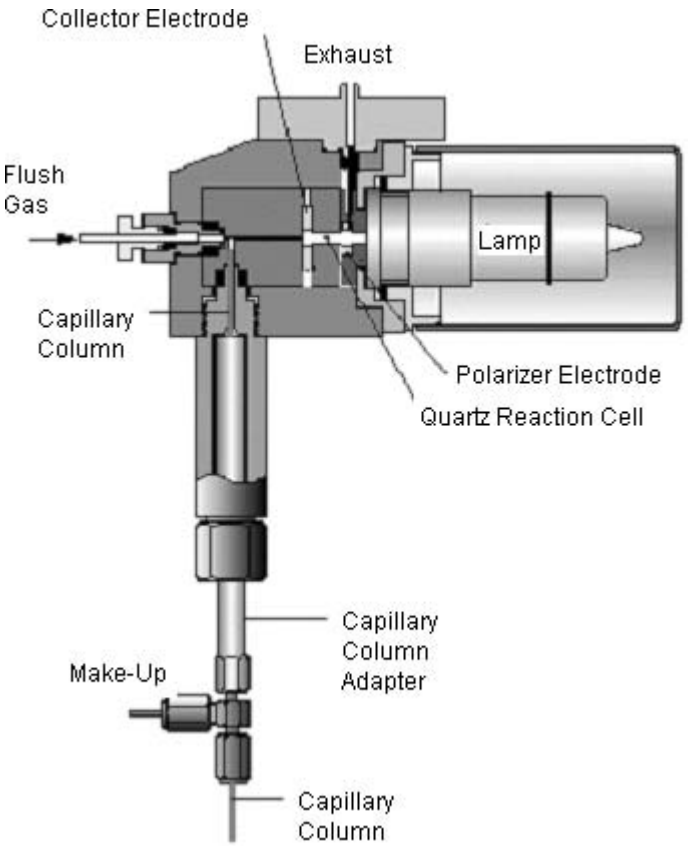

FIGURE 6.23 Schematic representation of a PID (illustration courtesy of Thermo-Finnigan).

process can be represented as

$$R + h\nu \rightarrow R^+ + e^- \tag{6.17}$$

The ions produced by such a process can be collected at an electrode where the current generated is proportional to the ionized species. Molecules with ionization potentials below the energy of the UV source can be ionized. The energy of the photon is also dependent on the source of the gas used as the UV emission source. Lower-energy lamps using krypton or xenon are more selective since they can excite a limited amount of compounds. Higher energy lamps, using argon or hydrogen, can provide for the excitation of a larger number of compounds. The more popular available UV lamps range from 8.3 to 11.7 eV.

Typically, the PID consists of a UV lamp mounted on a low-volume cell that is thermostatted (see Figure 6.23). The source and the ionization chamber must be separated by an optically transparent window. For high-energy photons, crystals of alkali and alkaline earth metal fluorides are used, since glass and quartz are not transparent to these photons. The crystal chosen depends on the emission spectrum of the gas used. The most popular lamp is the 10.2 eV hydrogen source with a magnesium fluoride window; this is because it exhibits the highest photon flux and, therefore, best sensitivity. The lamp emits UV radiation that ionizes only those species eluting from the gas chromatographic column whose ionization potential is below the energy provided by the lamp; it is not, however, as universal as the 11.7-eV argon lamp. To determine which lamp is most suitable for a particular experiment, ionization potential tables should be consulted. Compounds with higher ionization potentials than the source do not absorb the energy and they are not detected at the PID. Compounds that are routinely analyzed include aromatic hydrocarbons and organosulfur or organophosporus compounds.

6.7.3 Detector Characteristics

Freedman proposed an equation to account for the PID response (68):

$$i = I^0 F \eta \sigma N L[\mathrm{AB}] \tag{6.18}$$

where i is the PID response (ion current), I^0 is the initial photon flux, F is the Faraday constant, η is the efficiency coefficient of ionization, σ is the absorption cross section, N is Avogadro's number, L is the pathlength, and [AB] is the concentration of ionizable substance. The equation states that lamp energy and cell volume are independent variables that can be used to enhance the detector signal. A sensitivity study of a PID with a 10.2-eV lamp showed that sensitivity increases as the carbon number increases giving the following trends in sensitivity of (69)

Aromatics > alkenes > alkanes

Polycyclic > monocyclic

Branched > nonbranched

and that for substituted benzenes, ring activators increased the sensitivity, while ring deactivators decreased the sensitivity. Detection limits for aromatics, for example, are in the low picogram level. The sensitivity for benzene is about 0.3 C/g (Coulombs per gram) with a linear range of 10^7.

6.8 HELIUM IONIZATION DETECTORS

6.8.1 Introduction

Similar to the electron-capture detector, the helium ionization detector (HID) evolved from the argon ionization detector (see Section 6.5.1). In this case, metastable helium species are formed instead of the argon ones. The metastable helium species have energy of 19.8 eV, making it capable of ionizing molecules that the earlier argon ionization detector could not ionize. The ionization products formed are subjected to an electric field and the current change is measured. The metastable species do not necessarily need to be formed from the electrons induced by a radioactive source. An electrical discharge can also produce electrons that can be accelerated to collide with He to produce highly energetic metastable species.

6.8.2 Operating Principles, Design, and Characteristics

The HID is based on the principle that high-energy metastable helium species transfer their energy to sample molecules through collision, therefore inducing ionization. Traditionally, β-emitters (e.g., T^3H_2 and Sc^3H_3) have been used to initiate the ionization of helium gas, which eventually leads to the highly energetic helium species. More recently, radiation sources have been replaced by an electric arc to initiate ionization of helium; this has led to the name of helium discharge ionization detector (HDID). In this case, electrons and photons are produced by the electric discharge; therefore, it is probable that ionization occurs through a number of processes. The two most likely contributors to the ionization of molecules eluting from the chromatographic column are ionization due to the metastable helium species and photoionization due to the photons produced by the electric discharge.

The HID, with a radioactive source, most frequently makes use of the plane-parallel geometry, illustrated earlier in Figure 6.14b, or a symmetric coaxial configuration. Both designs have closely spaced electrodes minimizing internal volumes. Typical detector volumes range from 100 to 200 μL, which makes them suitable for use with conventional capillary columns. Purity of the carrier gas is a common problem with the helium ionization detector. Even with commercially available 99.9999% pure helium, the purity at the detector is not guaranteed due to leaks from the atmosphere. Placing the detector in a helium environment eliminates this problem. Stationary phase bleed is an important problem that may be attenuated with commercially available low-bleed columns, such as columns with the stationary phase bonded or immobilized on fused silica.

6.8.2.1 Helium Discharge Ionization Detector (HDID)

The HDID uses an electrical discharge to initiate ionization of helium. The ionization process does not depend solely on the metastable helium species; photoionization also plays a significant role. A diagram of the HDID is depicted in Figure 6.24. The detector is composed essentially of two cavities, one in which the gas discharge is initiated and the other in which the eluted molecules are ionized. The discharge is initiated when a flow of helium gas is passed through the cavity containing two electrodes with a potential difference of about 500 V. The gas from the discharge chamber passes to the second chamber, where helium carrier enters as the chromatographic column effluent. The discharge gas is set at a higher flowrate to maintain the upper cavity clean. As the energetic discharge gas enters the lower cavity, it provides the energy for ionization. The ions formed are collected at the bias electrode (at about 150 V) and amplified by the electrometer. The gases eventually leave the ionization chamber.

The response of the HID is very sensitive to impurities in the carrier gas and is dependent on the bias voltage used at the collecting electrode. For optimum performance, the use of high-purity helium gas is imperative (i.e., 99.9999%). The HDID has shown good sensitivity for the permanent gases (i.e., CH_4, O_2, Ar, N_2, H_2, CO, CO_2). LOD in the 10–20 ppb range is typical, with a linear range of up to 10^6.

6.8.2.2 Pulse Discharge Helium Ionization Detector (PDHID)

The PDHID is essentially the same configuration of the PDECD introduced in Section 6.5.4 and illustrated in Figure 6.18, with two minor changes. The bottom electrodes connections in Figure 6.18 (bias and collector) are interchanged and the gas dopant is not required. In principle, the PDHID relies, for the most part,

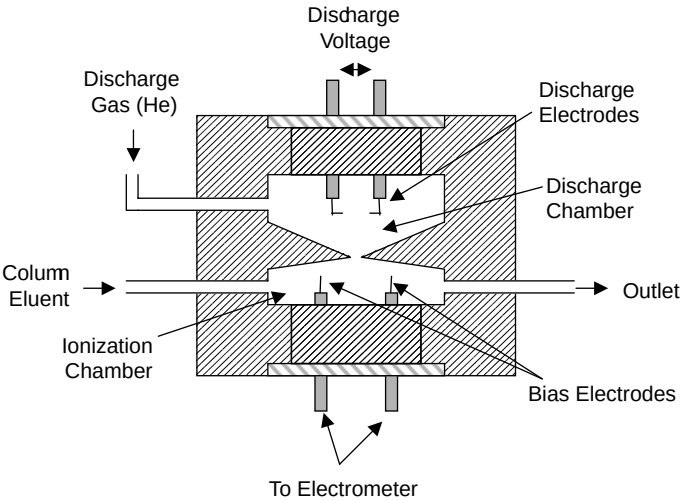

FIGURE 6.24 Diagram of the HDID.

on the radiation of excited helium species to effect ionization, making photoionization the principal mechanism of sample ionization. The ionization efficiency is below 1%; hence, it is considered a nondestructive, universal detector. As illustrated in Figure 6.18, the detector consists of two electrodes inserted into a cylindrical quartz insulator at about 2 mm apart, which produce a discharge of about 1 mm in diameter. There are two distinct zones in the detector: the discharge zone and the ionization zone. The ionization zone comprises the area of the three electrodes separated by sapphire insulators. For the discharge, helium is introduced at the top of the detector, while the sample in the carrier gas is introduced from the bottom. Introducing the sample and the discharge gas from opposite ends minimizes the contamination of the discharge electrodes by the components eluting from the chromatographic column. However, there is always the possibility of contamination with very high concentration of compounds entering the detector for extended periods of time.

The potential across the discharge electrodes is about 20 V and is pulsed at about 3 kHz. The species produced by means of the discharge (i.e., electrons, high-energy photons, and probably some metastable helium species) enter the reaction/ionization zone, mixing with the effluent of the chromatographic column and the solute molecules are ionized. The electrons thus produced are then focused toward the collector electrode via the two bias electrodes. The generated current is monitored through an electrometer. Adding small quantities of argon, krypton, or xenon to the discharge gas, results in changes of the emission profile of the discharge, providing additional photon energy for solute ionization. This allows the detector to function in a selective photoionization mode for determination of aliphatics, aromatics, amines, and other species.

With the exception of neon, which has an ionization potential higher than that of He, the PDHID response is universal. The linear response is over five orders of magnitude for both organic and inorganic compounds. It has been suggested as a replacement for the FID, particularly in situations where a flame and hydrogen may be hazardous. For the analysis of permanent gases, a packed column can be accommodated and the LOD are in the low-ppb (parts per billion) range. With a packed-column configuration, however, the linearity is three to four orders of magnitude.

6.9 FLAME PHOTOMETRIC DETECTOR

6.9.1 Operating Principles

The flame photometric detector (FPD) is based on the monitoring of the intensity of the light emission of species that have been excited in a flame. The components of the effluent of the chromatographic column are decomposed and then excited to a higher electronic state in a hydrogen-rich flame. These species emit light characteristic of the heteroatoms introduced into the flame. The intensity of the emission spectra is monitored by a photomultiplier tube (PMT). An optical filter in the radiation path is used to select the appropriate wavelength of light that

reaches the PMT. Although the FPD can respond to halogens, nitrogen containing compounds, tin, chromium selenium, tellurium, and boron by changing the flame conditions, it is used mostly for the monitoring of organic sulfur and organophosphorus species, for which the detector is generally considered selective.

The response mechanism of the FPD is not completely understood; this is the case because of the complex nature of the processes occurring in the flame. In general, it is accepted that for sulfur-containing compounds, they are combusted and interconverted to a series of sulfur species, such as HS, S, S_2, SO, H_2S, SO_2, and others that may include carbon–sulfur-containing species, depending on the flame chemistry. The energetically excited sulfur species are the result of collision reactions in the flame. In the case of phosphorus, the phosphorus-containing compounds are decomposed to PO species that will undergo collision reactions to produce excited HPO species. The excited species will decay to a lower energetic state, leading to light emission. One can refer to this phenomenon as a chemiluminescence process (see Section 6.10.1) in the flame.

6.9.2 Design

Flame photometric detectors can be single-burner (70) or dual-burner (71) as illustrated in Figure 6.25. In the original single-burner design, the effluent from the column is mixed with oxygen using the nitrogen carrier gas in a proportion similar to air. Excess hydrogen is added to the exterior of the burner tip. The diffusion flame is situated inside the burner tip to shield the PMT from a direct view of the flame. This design allows the emission of sulfur and phosphorus to occur above the shielded flame and in direct view of the PMT. Interferences

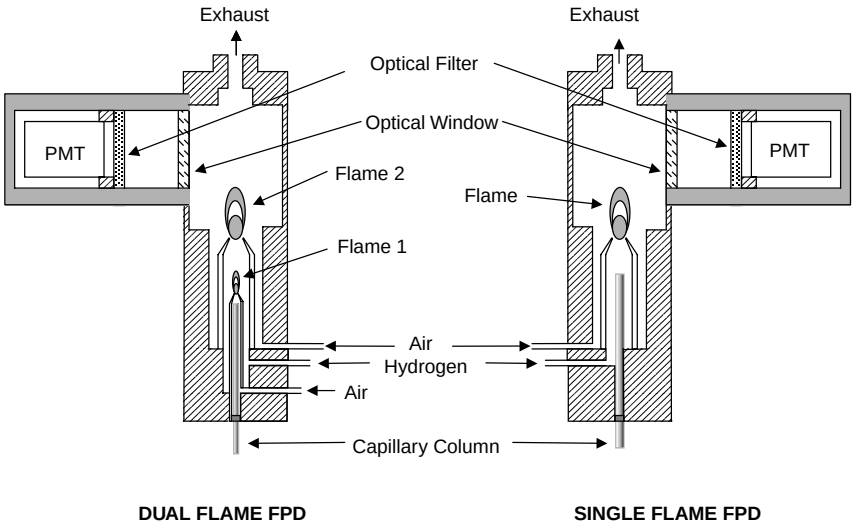

FIGURE 6.25 Schematic of the single flame FPD and the flames arrangement of the dual-flame FPD.

from hydrocarbons emitting light in the flame portion inside the burner tip are therefore not detected. The detector can incorporate two optical filters and two photomultiplier tubes for the simultaneous detection of sulfur and phosphorous. The 394-nm and 526-nm lines are typically monitored for sulfur and phosphorous, respectively.

Flame photometric detectors employ both diffusion and premixed flames. A premixed flame can have 40 times the noise of a diffusion flame, which is cooler and more suitable for phosphorus and sulfur. In the single-flame design, however, a large sample adversely affects the light emission by changing the temperature and geometry of the flame. Solvent flameout is another problem with the single-flame design. This occurs when the solvent peak elutes from the column and starves the flame of oxygen and effectively extinguishes it. This problem has since been eliminated by interchanging the oxygen and hydrogen inlets. In the dual-flame photometric detector, the column effluent is mixed with air while hydrogen is added at the base of the detector. The lower flame is responsible for combustion of the sample while light-emitting excited species are generated in the upper flame. With this design, the second flame can reignite the first in the event of solvent flameout.

A different detector design operates in a pulsed-flame mode instead of having the continuous flame (72), represented in Figure 6.26. This configuration is known as the pulsed-flame photometric detector (PFPD) and is based on a flame source and combustible gases in which a continuous flame cannot be sustained. Hydrogen and air are mixed with the effluent from the column, flowing continuously to a heated igniter. The gases are ignited and the flame propagates back to the combustion chamber and self-extinguishes after the combustible mixture is burned. The continuous flow of gases removes the combustion products and provides for reignition of the flame. This process is repeated at about 4 Hz. Emission

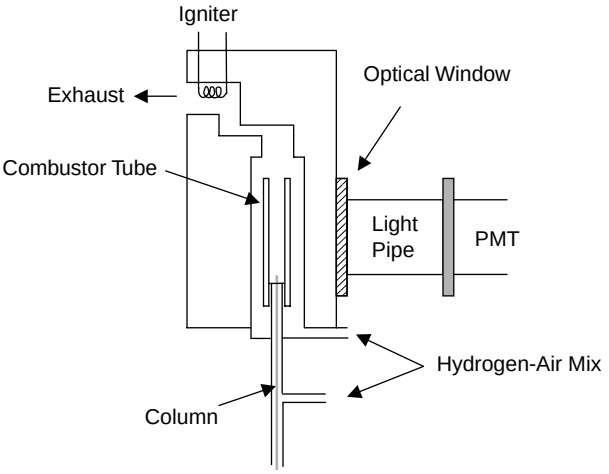

FIGURE 6.26 Schematic of the pulsed- FPD.

from carbon and the combustion flame are complete in a few milliseconds; after that, several species give a delayed emission lasting up to 20 ms. The emission is filtered and detected by a PMT. This approach eliminates flame background and hydrocarbon interferences that can limit detectability (see text below). Another advantage is that the detector is not limited to sulfur and phosphorous containing compounds; many other species can be determined with good detectability.

6.9.3 Performance Characteristics

6.9.3.1 Noise and Detection Limits

Photomultiplier noise increases with temperature, so the temperature of the detector should be set just high enough to avoid condensation of the high-molecular-weight compounds in the detector. The operating range of the FPD is usually 150−275°C because of the proximity of the photomultiplier tube. The minimum detectable level using the FPD depends on operating conditions of the detector and its geometry and the PMT; the MDL is approximately 0.5 pg/s for phosphorus and on the order of 50 pg/s for sulfur. However, the detectabilities for a commercially available PFPD are $<$0.1 pg/s for phosphorus (methylparathion), $<$1 pg/s for sulfur (methylparathion), and $<$10 pg/s for nitrogen (nitrobenzene).

6.9.3.2 Sensitivity and Dynamic Range

The sensitivity of the FPD is dependent on the intensity of the light emitted by the excited species, which increases with decreasing flame temperature. Therefore, using carrier gases with high thermal conductivities, such as helium or hydrogen, increases sensitivity by decreasing the flame temperature. The sensitivity of the FPD also increases with excess hydrogen in the diffuse flame. Unfortunately, the excess hydrogen makes the flame unstable and easily extinguishable during solvent elution. The response in the phosphorus mode is linear over a 10^4 range. The sulfur response, however, varies such that the square root of the response is proportional to the concentration and is linear on a log−log scale over three orders of magnitude.

6.10 CHEMILUMINESCENCE DETECTORS

6.10.1 Introduction

Chemiluminescence is the emission of light by the virtue of chemical reactions. An energetically excited species is produced in a chemical reaction. Such species can decay to a lower state of energy by emitting light, as shown below

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C}^* + \mathrm{D} \tag{6.19}$$

$$\mathrm{C}^* \rightarrow \mathrm{C} + h\nu \tag{6.20}$$

where A and B represent reactants, C^* is a reaction product in an excited state, and C represents the species after the energy has been dissipated; D is another

reaction product, and $h\nu$ represents the emission of a photon. Alternatively, the excited species can transfer the energy to another molecule that can then decay emitting light. The reaction occurs on a time scale such that the production of light is essentially instantaneous. The intensity of the light emitted is proportional to the concentration of the species involved in the chemical reactions. In addition the radiation emitted offers very low background.

For chemiluminescence detection in GC, the analytes eluting from the column are directed into a reaction chamber. The resulting $h\nu$ is detected by a PMT. To obtain the minimum amount of energy loss to gas phase collisions, the chamber is maintained at low pressures of approximately 1 Torr. This allows the majority of the energy produced to be used for excitation of analyte molecules.

6.10.2 Sulfur Chemiluminescence Detector

The sulfur chemiluminescence detector (SCD) for GC was developed by Benner and Stedman and is based on the formation of sulfur monoxide from sulfur containing compounds by combustion in a reducing hydrogen/oxygen flame (73). The effluent from a column enters a combustion tube with a stainless steel burner maintained at 800°C. The combustion process, however, can achieve temperatures of 1800°C. The products of combustion are transferred to a reaction cell under vacuum, and ozone is added to the reaction cell, resulting in a chemiluminescence reaction.

$$\text{S-containing compound} + \text{O} \rightarrow \text{SO} + \text{other products} \tag{6.21}$$

$$\text{SO} + \text{O}_3 \rightarrow \text{SO}_2 + \text{O}_2 + h\nu \tag{6.22}$$

The emitted light is monitored by a PMT. For a commercially available SCD, the reaction cell operates at pressures of about 5–10 Torr while the burner operating pressure is in the range of 130–275 Torr. Typical flow rates for the gases are 6–12 mL/min for oxygen (15–40 mL/min if air), and 75–100 mL hydrogen. The ozone is introduced into the reaction cell at about 50 mL/min. The SCD linearity is about 10^5 with detectability of 0.5 pg/s.

A flameless sulfur chemiluminescence detector has also been described (74). The design uses an externally heated ceramic assembly that is operated at low pressure under the necessary fuel-rich conditions but they are out of the flammability limits of hydrogen in air. The hydrogen and air are mixed as the effluent reaches a high-temperature zone. This results in partial oxidation before the effluent reaches the highest temperature zone. It utilizes combustion at low pressure, which is thought to increase the production of sulfur monoxide. The flameless system reduces the effect of column bleed and shows improved detectability over conventional SCD by about one order of magnitude.

6.10.3 Nitrogen Chemiluminescence Detector

A nitrogen-specific chemiluminescence detector exists that is very similar to the SCD. The effluent from a column enters a combustion tube with a stainless-steel

burner, and the subsequent nitrous oxide product is reacted with ozone to produce the luminescence as shown in the reactions below:

$$R - \mathrm{N} + \mathrm{H_2} + \mathrm{O_2} \rightarrow \mathrm{NO} + \mathrm{CO_2} + \mathrm{H_2O} \tag{6.23}$$

$$\mathrm{NO} + \mathrm{O_3} \rightarrow \mathrm{NO_2} + h\nu \tag{6.24}$$

The emitted light is proportional to the amount of nitrogen in the sample. The detector responds to nitrogen linearly with equimolar response and linearity of 10^4. The LOD for nitrogen is about 5 pg/s. The detector responds not just to organic nitrogen compounds but also to ammonia, hydrazine, hydrogen cyanide, and NO_x. The operating conditions are similar to those of the SCD.

6.11 ATOMIC EMISSION DETECTOR

Plasmas are commonly used excitation sources in atomic emission spectroscopy. Typically, a plasma is an electrical discharge in which a gas is energized by means of direct current or high-frequency electromagnetic fields. The electrical discharge is a composite of a highly energetic gas containing different species of the supporting gas (e.g., free atoms, molecules, ions). There are three major plasma sources: the inductively coupled plasma (ICP), the direct current plasma (DCP), and the microwave-induced plasma (MIP). Plasma sources provide high atomization efficiencies and high degrees of excitation. A portion of this energy is transferred to the sample, once it enters the plasma, allowing atomization and excitation to take place.

Plasma sources have been used for element-specific detection in gas chromatography. However, the plasma source most widely employed for gas chromatographic detection is the MIP, which has been the only plasma source that developed into a commercial gas chromatographic detector. Since the operating principle of the MIP is atomic emission spectroscopy, the detector has been termed *atomic emission detector* (AED).

The use of an MPD as a gas chromatography detector was first reported by McCormack et al. (75). The modern AED uses high-purity helium as a gas to sustain the plasma within a discharge tube inside a cavity were the microwave power is concentrated; operational powers are about 50–450 W. Helium is introduced as a carrier gas and as a makeup gas. The highly energetic helium species produced in the plasma leads to excitation and high spectral intensities for many elements, including nonmetals. As solutes emerge from the chromatographic column, they are directed into the microwave powered plasma contained in the discharge tube positioned inside the cavity, where the solutes are decomposed into their atomic components and excited by the high-energy plasma. The excited species emit radiation that is characteristic of the excited atomic species. The emitted light is spectrally separated into individual lines using a reflection grating in a multiple wavelength diode array spectrophotometer, providing optical resolution, hence the selective element detection of sample components. Carbon buildup in the

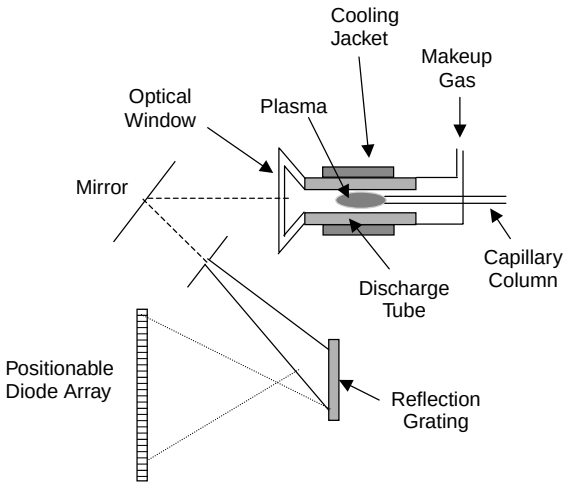

FIGURE 6.27 Schematic representation of the AED.

discharge tube is prevented by doping the plasma with low levels of oxygen or nitrogen. Multiple elements can be monitored simultaneously. This allows for empirical formulas for assistance in molecular structure elucidation. A schematic representation of the AED is shown in Figure 6.27.

The instrumentation available operates at atmospheric pressure and incorporates a water-cooled discharge tube to maximize the signal to background ratio. Ultrapure helium and a clean, leak-free chromatographic system are essential since the spectra of impurities can be quite intense. The sensitivity of the AED is quite good with minimum detectable limits of 0.1 pg–1 ng depending on the element. The major strength of the AED is its ability to simultaneously determine the atomic emissions of many of the elements in components exiting the gas chromatographic column.

6.12 OTHER DETECTORS

6.12.1 Hall Electrolytic Conductivity Detector

Coulson made the first commercial gas chromatographic detector based on the electrolytic conductivity of ionic species in water (76). Analytes that eluted from the column were oxidized or reduced catalytically as shown in the relationships below to form an ionic species that was transferred to a stream of deionized water for detection:

$$\text{S (X, N) containing organic} + O_2 \rightarrow SO_2(X_2, NO_x) + CO_2 + H_2O \quad (6.25a)$$

$$SO_2 + 3H_2O \rightarrow 2H_3O^+ + SO_3{}^{2-} \quad (6.25b)$$

$$SO_3 + 3H_2O \rightarrow 2H_3O^+ + SO_4{}^{2-} \quad (6.25c)$$

$$N_2O_3 + 3H_2O \rightarrow 2H_3O^+ + 2NO_2^- \quad (6.25d)$$

$$CO_2 + 3H_2O \rightarrow 2H_3O^+ + CO_3^{2-} \quad (6.25e)$$

$$\text{S (X, N) containing organic} + H_2 \rightarrow H_2S(HX, NH_3) + CH_4 + H_2O \quad (6.26a)$$

$$H_2S + 3H_2O \rightarrow 2H_3O^+ + S^{2-} \quad (6.26b)$$

$$HX + H_2O \rightarrow H_3O^+ + X^- \quad (6.26c)$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (6.26d)$$

After detection, the water was passed through ion exchange resins to remove the ions and then circulated back through the system. The oxidative mode is ineffective due to carbon dioxide being dissolved in the liquid stream. Hall developed an improved design to detect picogram quantities of compounds containing halogens, sulfur, or nitrogen.

There are three principal detection modes in commercial detectors: halogen mode to detect HX, sulfur mode for detecting SO_2 or SO_3, and nitrogen mode for detecting NH_3. The detector response depends on the reaction conditions, the solvent, the pH, and the use of a postreaction scrubber. A schematic representation of a commercially available detector unit is shown in Figure 6.28.

In the halogen mode, a nickel reaction tube with hydrogen reaction gas is used at a temperature of 850–1000°C. This converts compounds containing halogens into their corresponding hydrogen halide and other non-halogen-containing products. The conductivity solvent, n-propyl alcohol, can dissolve the halogen-containing products, but not those non-halogen-containing species. The dissolved halogen products change the electrolytic conductivity of the solvent, which is then measured. In the halogen mode, sulfur-containing compounds are converted to H_2S, while in the nitrogen mode nitrogen-containing compounds are converted to NH_3, both of which are poorly ionized in propanol.

The nickel reaction tube, with air as the reaction gas at 850–1000°C, is used in the sulfur mode to convert the sulfur-containing compounds to SO_2. Methyl alcohol containing a small amount of water is used as the conductivity solvent. In this mode, nitrogen-containing compounds are converted to N_2 and some nitrogen oxides, which show little or no response. Any halogen-containing compounds are converted to HX and must be removed with a postreaction scrubber containing strands of silver wire.

In the nitrogen mode, the nickel reaction tube with hydrogen reaction gas is operated at 850–1000°C. Water containing a small amount of organic solvent is the conductivity solvent and fully ionizes the NH_3. Because trace quantities of CO_2 can neutralize the NH_3, care must be taken to prevent its permeation into the solvent. In this mode, both HX and H_2S can be formed and a postreaction scrubber consisting of a length of coiled tubing containing several strands of quartz thread coated with KOH is used.

Two types of detectors cells exist commercially, the dynamic reservoir cell and the mixed-phase cell. The dynamic reservoir cell separates the gas and liquid streams before measurement of conductivity, while the mixed-phase cell allows

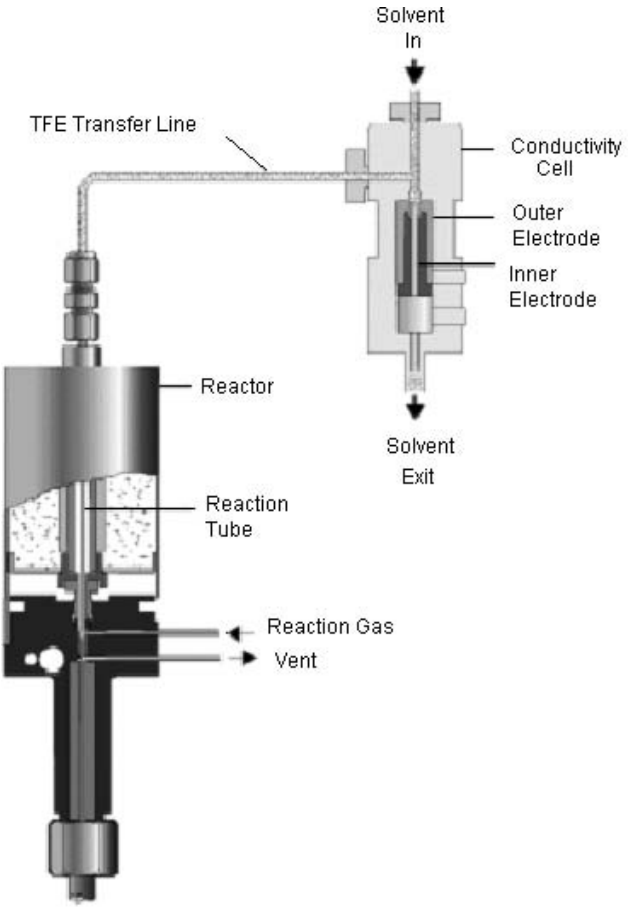

FIGURE 6.28 Schematic of the Hall electrolytic conductivity detector (illustration courtesy of ThermoFinnigan).

both the gas and liquid phases to pass through the measurement zone. Cell optimization is achieved by adjusting the reactor temperature, reactant gas flowrate, solvent flowrate, composition and surface area of the nickel catalyst, and cell voltage. In the halogen mode the detector detectability is <10 pg (heptachlor) with a linearity of 10^6. In the sulfur nitrogen mode, the linearity is just 10^3 with detectability of about 20 pg (ethion) and <10 pg, for sulfur and nitrogen, respectively.

6.12.2 Ultrasonic Detector

The ultrasonic detector is a universal detector, with a broad dynamic range, good sensitivity, and a wide choice of carrier gases. Illustrated in Figure 6.29, this detector propagates sound waves at one transducer and receives them at another.

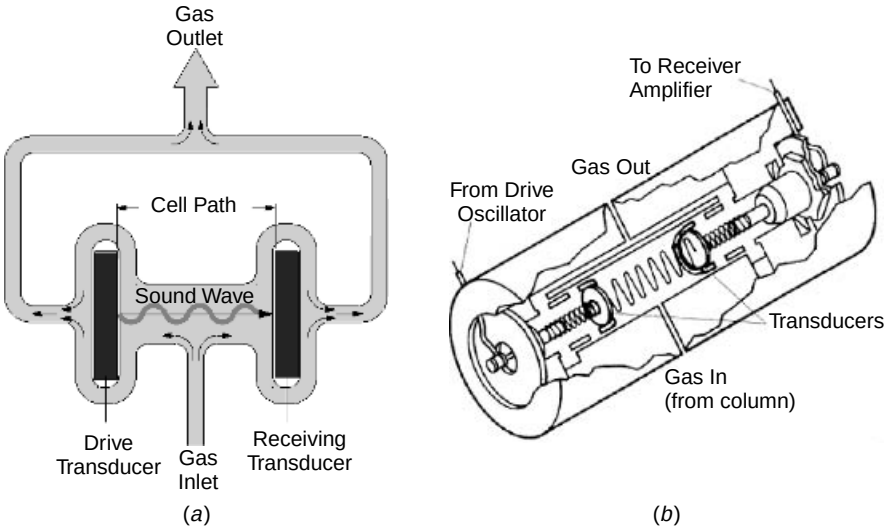

FIGURE 6.29 (a) Phase shift measurement and (b) cross section of the ultrasonic detector (illustration courtesy of ThermoFinnigan).

A phase meter monitors the signal received, which is sensitive to any changes. The changes in the phase angle ϕ with the introduction of a component in the mobile phase are expressed in the following mathematical relationship

$$\phi = 180sf\left(\frac{M_1}{RT_{\gamma 1}}\right)^{1/2} n\left[\frac{M_2}{M_1}\left[\frac{1+\left(\frac{C_{p2}}{C_{p1}}\right)\gamma_1}{(\gamma_2-1)}\right]-1\right] \tag{6.27}$$

where ϕ is the degrees of phase change, s is the sound pathlength (cm), f is the frequency (cycles/s), M_1 is the molecular weight of the carrier gas, M_2 is the molecular weight of the sample gas, R is the gas constant, T is the absolute temperature of gas, γ_1 is the specific-heat ratio of the carrier gas, γ_2 is the specific-heat ratio of the sample gas, n is the mole fraction of sample, C_{p1} is the gram specific-heat ratio of carrier gas at constant pressure, and C_{p2} is the gram-specific heat ratio of sample gas at constant pressure.

From Equation 6.25, it is apparent that the detector is sensitive to pressure. A backpressure must be maintained in the cell sufficient to support propagation of the waves. For hydrogen, 65 psi is necessary, but for helium, only 10 psi is required. Good temperature control is necessary, and at the lower limits of detection 10^{-3}–10^{-4}°C is required. Flowrate variations within the range of 30–80 mL/min do not affect response, but below 10 mL/min, the change is exponential. The separation distance of the transducers is critical and must be optimized for the particular carrier gas. The cell volume affects the resolution in

capillary GC only. The linear dynamic range of the detector is over six orders of magnitude. Use of this detector is restricted to permanent gases and low boiling compounds since the detector does not perform well at elevated temperatures above 200°C.

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CHAPTER SEVEN

Techniques for Gas Chromatography/ Mass Spectrometry

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7.1 INTRODUCTION

Gas chromatography/mass spectrometry (GCMS) combines the power of high-resolution separation of components with very selective and sensitive mass detection. Since the early 1990s high-cost research-grade features such as chemical ionization (CI) and tandem mass spectrometry/mass spectrometry (MS/MS) have become commercially available for low-cost benchtop GCMS systems. By incorporating automation, miniaturization, and simplification into its design, GCMS has evolved since the early 1970s such that it has a broad range of applications. For example, GCMS can be used to delineate steroid-related disorders in children and adults (1) as well as to study the composition of planetary atmospheres (2). Since a relatively small range of compounds are amenable to GCMS analysis as compared to liquid chromatography/mass spectrometry (LCMS) technology, LCMS is currently growing at a faster pace than GCMS. However, GC/MS has many attributes. For example, a major strength of GCMS is its ability in identification of unknown compounds through the use of established and extensive chemical and electron ionization libraries.

7.1.1 Brief History of Gas Chromatography/Mass Spectrometry

Mass spectrometry and gas chromatography have a long and interesting history. Mass spectrometry was discovered around the turn of the century when Thomson (3) obtained mass spectra of compounds such as oxygen and nitrogen. Aston (4,5) and Dempster (6) further developed it through the early twentieth century where their studies focused on using the technique to determine elemental isotopes. Although Thomson recognized its potential for chemical analysis very

early, it was not until the 1950's with the availability of commercial instruments that MS was first used diagnostically in the oil industry. Mass spectrometers must operate at low pressures, typically in the range 10^{-5}–10^{-7} Torr (1 Pa = 0.0075 Torr), to minimize ion–molecule collisions. This requirement was the major obstacle for chromatographic coupling, both GC and liquid chromatography to mass spectrometers. James and Martin (7) developed gas–liquid chromatography in 1952, and open tubular columns were developed by Golay in 1958 (8). In 1957, Holmes and Morrell demonstrated the first coupling of gas chromatography with mass spectrometry (9). In their experiment, they continuously monitored the effluent from a gas chromatographic column with a mass spectrometer and an oscilloscope. The oscilloscope could display any 16-mass-unit portion of the mass spectrum. Since mass spectrometer vacuum systems cannot directly accommodate the higher carrier-gas flow required for packed-column GC, various interfaces were developed over the years to separate carrier gas from samples after gas chromatography, thus reducing the pressure from the effluent flow before it entered the mass spectrometer (10). The first commercial packed-column GCMS systems were the Swedish LKB magnetic instrument in the mid-1960s, the Finnigan quadrupole instrument, and the Hewlett-Packard quadrupole instrument in the early 1970s. The advent of capillary GC simplified the coupling of these techniques, since modern mass spectrometric vacuum systems easily accommodate the lower carrier-gas flow rates, eliminating the lower efficiency interfaces required for packed-column GCMS. In the early 1970s, differentially pumped mass spectrometers for online capillary GCMS were developed. McFadden authored the first book on GCMS (11). With the introduction of data-processing computers (12) and the invention of fused-silica GC columns in 1976 by Dandeneau and Zerenner (13), modern commercial GCMS instruments were quickly applied to a large array of analytical applications (13).

7.1.2 Scope of Chapter

While the skill needed to operate GCMS equipment is much less today than in the early 1990s, due primarily to complete microprocessor and software control of the instrument, understanding the underlying principles of chromatography and mass spectrometry are still required to use GCMS optimally. The principles of gas chromatography are described throughout this book. In this chapter, the principles of MS and GCMS are discussed. For more comprehensive treatments, readers are directed to several volumes dedicated exclusively to GCMS and its applications (10,11,15–27).

7.1.3 Overview of Gas Chromatography/Mass Spectrometry

The schematic diagram showing the major components of a typical capillary GCMS system is presented in Figure 7.1. The gaseous effluent from the chromatograph is directed through the transfer line into the ion source. The vaporized analytes are then ionized, producing molecular and/or fragment ions, which are

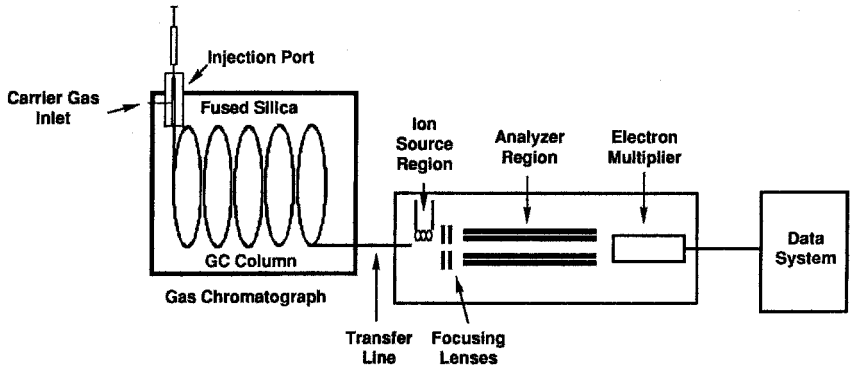

FIGURE 7.1 Schematic diagram of a typical gas chromatograph/mass spectrometer system. Gaseous analytes eluting from the chromatograph are directed into the spectrometer ion source where they are ionized. The ions produced are separated according to their m/z values and detected.

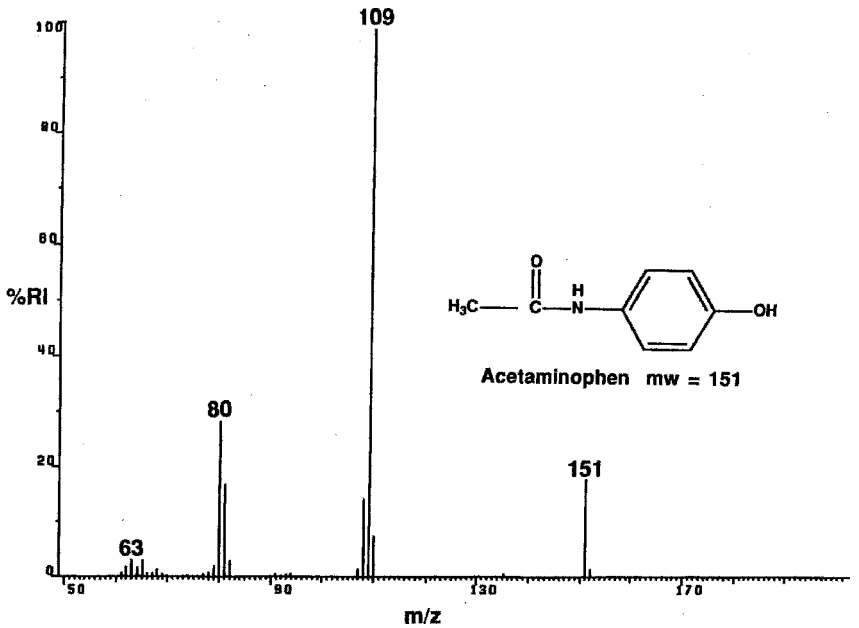

FIGURE 7.2 The mass spectrum of acetaminophen. Spectra are normally plotted with the mass-to-charge ratio (m/z) on the x axis (abscissa) and the relative intensity (%I) on the y axis (ordinate). Since the majority of ions are produced with only one charge ($z = 1$), the m/z is equal to the mass of the ion. Note that the m/z is a dimensionless unit. The intensity of a peak is expressed as a percent of the base peak. The peak at m/z 151 represents the intact acetaminophen molecule and is referred to as the *molecular ion* ($C_8H_9NO_2$). The largest peak in a particular spectrum is called the *base peak*. The peak at m/z 109 is the largest fragment ion produced and is referred to as the *base peak of the spectrum*. This terminology carries throughout all mass spectrometry.

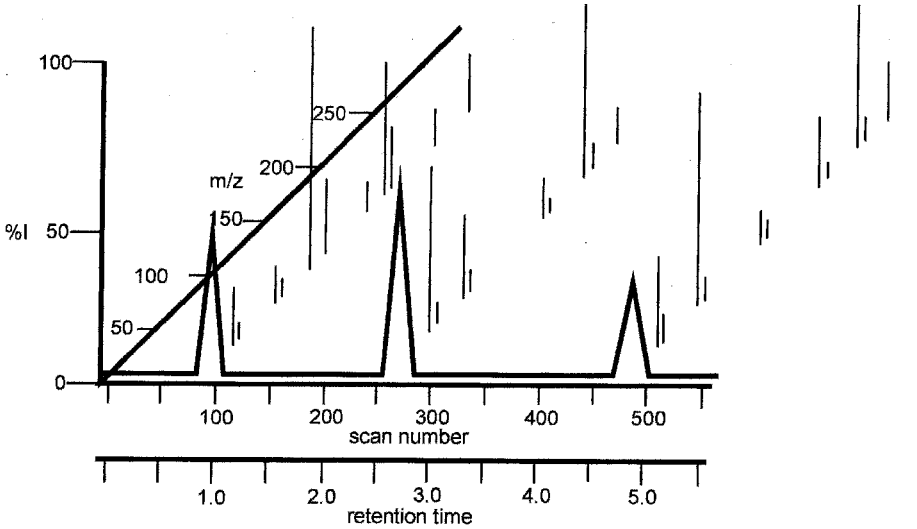

FIGURE 7.3 The data generated, from a GCMS experiment, represented here as a three-dimensional plot of scan number (time) versus mass/charge (m/z) versus relative intensity (%I).

then mass resolved utilizing a mass filter and detected. The resulting mass spectrum is displayed as a plot of the relative intensity of these ions versus their mass-to-charge ratio (m/z). Since most ions produced are singly charged, their m/z values are indicative of their masses. Atomic mass units are defined as daltons (μ). A typical mass spectrum of the common analgesic acetaminophen is shown in Figure 7.2. As the gas chromatographic separation proceeds, the mass analyzer is repeatedly scanned. The ion intensities for all m/z values for each scan can then be summed to generate a chromatographic trace commonly called a *total-ion current* chromatogram. This is illustrated in Figure 7.3. Searching libraries of mass spectra enhances the interpretation of the data. GCMS usually requires analytes that are volatile and thermally stable. This is the main limitation of GCMS since only approximately 10% of all organic compounds are suitable for direct GCMS analysis. Volatile and more thermally stable derivatives of many substances have been prepared for analysis, extending the utility of the technique (18).

7.2 GENERAL GAS CHROMATOGRAPHY/MASS SPECTROMETRY CONSIDERATIONS

GCMS methods are routinely used for qualitative identification of unknown compounds and the accurate quantitative determination of these compounds. In this section, the "GCMS journey" from sample preparation to data presentation will unfold. Practical aspects of each subtopic will be covered along the way.

7.2.1 Sample Preparation

In preparing samples for GC/MS, one simple fact must be kept in mind, namely, that everything injected onto the gas chromatographic column will be deposited into the mass spectrometer with the exception of those sample components which remain in the injection port or on the column. For volatile components this is not a concern as they are pumped away by the spectrometer vacuum system without consequence, but semivolatile materials may deposit in the ion source of the spectrometer with resultant loss of sensitivity, increased maintenance, and other unfavorable results. It is not uncommon for normal column bleed to eventually degrade system performance. For particularly valuable samples, such as metabolite extracts, biological samples, or other samples obtained through extensive effort, the contamination threat must be tolerated as the cost of analysis. However, if sample cleanup is possible without significant sample alteration, then a reasonable effort should be made to prevent contamination of the spectrometer.

Several methods have appeared in the literature concerning preconcentrating sample preparation. These include liquid–liquid extraction (LLE) (28), solid-phase extraction (SPE) (29), and solid-phase microextraction (SPME) (30) techniques. Novel procedures using affinity chromatography SPE techniques have been used for sample preparation in quantitative GCMS methods (31). Various natural and synthetic compounds including nitrosylated and nitrated proteins, arachidonic acid derivates, steroids, drugs, and toxins, have been developed as novel solid-phase extraction (SPE) materials. These SPE techniques have been shown to be suitable for selective extraction of analytes from various matrices. Online SPE GCMS methods for water analysis (32) and offline methods for drug determination in biological fluids using a commercially available robotic system have been reviewed (33). For additional discussions of the sample preparation techniques, see Chapter 11.

Inorganic nonvolatiles can be removed by such methods as ion-exchange or extraction. Polar, organic, nonvolatiles can be removed using silicagel or Florisil. These methods require caution, to avoid inadvertent loss of analyte.

Solvent selection is also important in GCMS. Since the mass spectrometer is typically scanned over a wide mass range during data acquisition, it is important to minimize the possibility of interference peaks. This is accomplished by choosing a solvent that does not generate peaks in the mass range of interest. Methanol is a good solvent choice when detecting components with MW $< 100\ \mu$ and detecting peaks close to the solvent front, since it has a low molecular mass of only $32\ \mu$. Solvents such as chloroform or methylene chloride, with their higher masses ($118\ \mu$ and $84\ \mu$, respectively), should be avoided in these cases. Typical spectra of these three solvents are shown in Figure 7.4.

7.2.2 Chemical Derivatization

In GCMS, chemical derivatization of the sample molecule often improves peak symmetry, volatility and thermal stability for gas chromatographic separations and can afford improved selectivity and detection limits for mass spectral

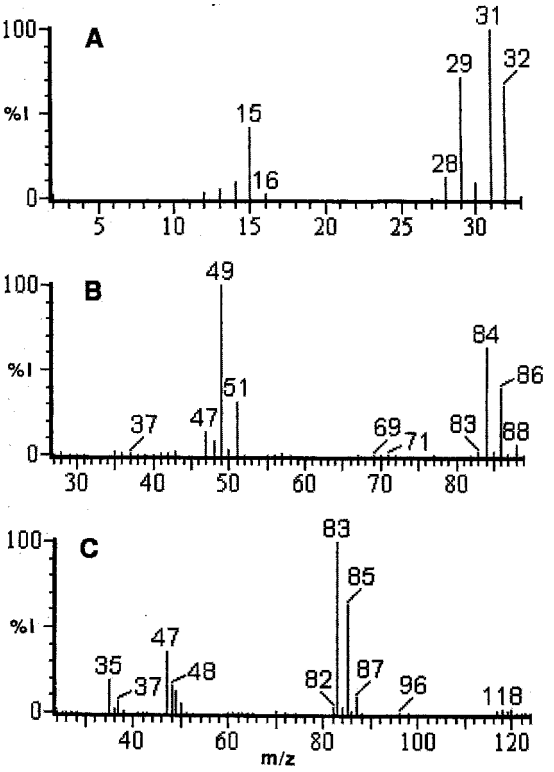

FIGURE 7.4 Electron ionization mass spectra of (a) methanol; (b) methylene chloride; (c) chloroform.

analyses. The most common form of derivatization of an active hydrogen atom (–OH, $–CO_2H$, $–NH_2$, –NHR, –SH) in a sample molecule is replacement of this atom with a trimethylsilyl (TMS) group. Many reagents are available for preparation of TMS derivatives such as *N*,*O*-bis(trimethylsilyl)acetamide and bis(trimethylsilyl) trifluoroacetamide. Since each TMS group adds 73 μ to the analyte mass, depending on the number of active hydrogens, this could add significantly to the mass of a compound and put it out of range of the spectrometer's upper mass limit. In such case, a fragment may have to be used to characterize the derivative.

The reaction of diazomethane with carboxylic acids to form methyl esters and with phenols to produce aromatic methyl ethers is easy to perform. In addition to providing a more volatile derivative, this method can be used diagnostically to verify the presence of these compounds as the observed mass will increase by 14 for each reactive OH group. This derivatization is frequently used in drug metabolism studies, since polar acids and phenols are often produced by the biotransformation of pharmaceutical agents. For more details on chemical derivatization, the reader is referred to the literature (18,34,35).

7.2.3 Chromatography

Although considerable work had been done in the past with packed columns, they are now rarely utilized in GCMS because of lower chromatographic and transfer efficiencies. Capillary column GCMS, with its simpler interface design and higher chromatographic efficiency, is typically preferred in all areas of analysis and will be exemplified in this discussion.

The limiting factor with respect to column selection is the maximum flowrate that can be accommodated by the spectrometer vacuum system. This is usually no more than 1 mL/min for standard instruments. This limits column diameters to either 0.25 mm or 0.32 mm i.d. Higher flow rates are possible with certain instruments according to design, pumping capacity and application, but columns of 0.53 mm i.d. are usually too large in physical size and/or required carrier-gas flow.

Either split, splitless, or on-column injections can be used in GCMS. Split injections are usually avoided in cases where trace-level components are being analyzed. Splitless or on-column injections are preferred for trace component analysis. Often splitless injections (septum purgeoff) are made with split injection port liners that are packed with a small amount of adsorbent to trap nonvolatile, polar materials that could contaminate the column and/or the mass spectrometer. For the same reasons, a retention gap is recommended for on-column injections, as the initial column section can be replaced with little effect on the chromatogram. When using a retention gap, however, care must be taken to ensure leaktight connections since a small air leak can have greater consequences for the mass spectrometer than for other detectors.

In general, there are no significant limitations on chromatographic operational parameters in GCMS, with the exception of flowrates as previously discussed. A few specific minor recommendations include failure to maintain columns near their maximum operating temperatures for extended periods as the increased column bleed may degrade spectrometer performance. In addition, it is usually best to condition columns before connecting them to the spectrometer. Split injections should be utilized when possible to avoid solvent contamination of vacuum pumps and prevent premature filament failure. Extended use of corrosive carrier gases, such as ammonia, should be avoided when possible.

7.2.4 Gas Chromatography/Mass Spectrometry Interfaces

Prior to the introduction of capillary columns in GC, it was necessary to eliminate the larger volumes of carrier gas eluting from the chromatograph prior to introduction into the mass spectrometer. Various interfaces were developed for packed column GC. These included the jet, membrane and effusion separators, to name only a few. The purpose of all of these devices is to eliminate most of the carrier gas, thereby enriching the analyte concentration. Unfortunately, in many cases, a large percentage of the analyte was removed as well and efficiencies in the 20–50% percent range were common. Since these devices are no

longer in widespread use, the details of their design and operation will not be covered here. Comprehensive discussions of these are available to the interested reader (10).

Capillary GC enables direct coupling of the chromatographic column to the mass spectrometer since flowrates are substantially reduced, typically from about 30 to 1 mL/min. The main requirement for these interfaces is that a constant temperature be maintained across the entire length from oven to ion source with no "cold spots," which may cause peak broadening or trapping of high boiling components. As simple as this requirement may seem, it is sometimes difficult to achieve because of ion source geometry, system configurations, and other variables.

7.2.5 Temperature Problems

As in gas chromatography, thermal degradation of components can occur in GCMS. This degradation is frequently catalyzed by active sites somewhere in the chromatographic system.

The injection port is normally constructed with a replaceable glass liner. The silanol groups normally present on glass surfaces can cause degradation of sample components. An example of thermal degradation is shown in Figure 7.5, in which a fructose derivative (topiramate) being developed as an anticonvulsant was analyzed by GCMS (36). As the injection port temperature was increased, a new peak was detected that was identified as a thermal degradant. It is worthy to note that this degradation was usually detected after repeated injections at elevated temperatures. Typically, injection port liners are deactivated with silanizing reagents that convert silanol groups to trimethylsilyl ethers. The use of on-column injections is also recommended to prevent thermal degradation, since the fused-silica capillary column is coated with liquid phase and fewer active silanol groups are present and injections are usually performed at lower temperatures.

It is also possible for thermal degradation to occur during separation on the column itself. Deactivation of the column can be performed in a similar manner by injection of a silanizing reagent prior to analysis. Since the interface may be operated at a higher temperature than the column oven, it can also be the location of thermal degradation. Lowering the interface temperature can minimize this problem.

Finally, the mass spectrometer ion source itself can cause degradation since the heated metal surfaces that the vaporized analyte molecules are subjected to, can act as a catalyst. Again, decreasing the source temperature can minimize this problem if possible. In GC with other detectors, the identity of the degradant is not generally known without running authentic standards, trapping of peaks, and matching retention times. However, with the additional molecular weight and structural information provided by the mass spectrometer, degradation products can frequently be identified during analysis. The nature of this degradation can often lead to specific chromatographic remedies.

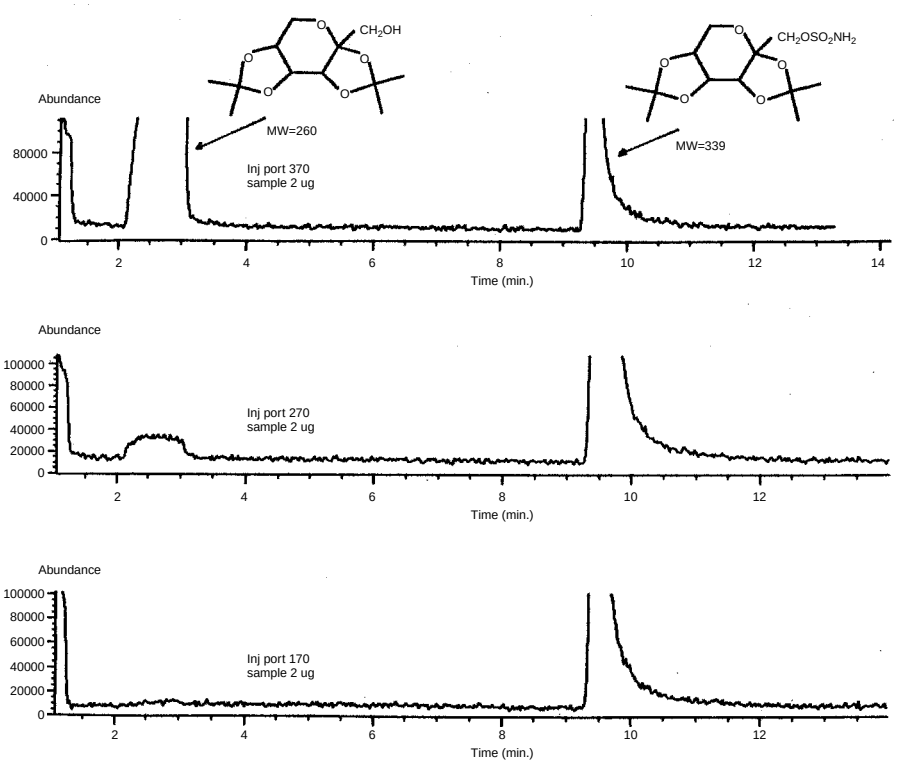

FIGURE 7.5 The total-ion current (TIC) chromatograms above demonstrate the thermal decomposition observed during gas chromatographic analysis of topiramate [molecular weight (MW) = 339 μ]. As the injection port temperature is increased from 170 to 370°C, decomposition to the alcohol derivative (MW = 260 μ) is observed. (Masucci and Caldwell, unpublished data).

7.2.6 Ion Sources

The purpose of the *ion source*, as the term implies, is to provide the energy necessary to ionize the analyte molecules, while being maintained at a temperature high enough to prevent analyte condensation. In addition, electrostatic focusing lenses are usually included to accelerate the ions and collimate the ion beam. The two types of ionization normally used in GCMS are electron ionization (EI) and chemical ionization (CI). The specifics of their operation are covered in Sections 7.3 and 7.4, respectively.

A schematic diagram of a typical EI source is shown in Figure 7.6. It is important that the capillary column extend as close as possible to the ionization region without obstructing the ion or electron beams. This will maximize analyte transport into the ion source and minimize the possibility for thermal degradation.

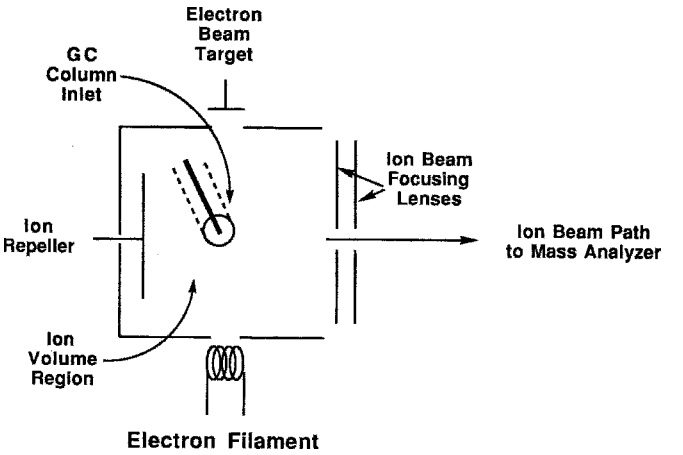

FIGURE 7.6 Diagram of an electron ionization source.

7.2.7 Mass Analyzers

As ions leave the source, they enter into the mass analyzer (mass filter), where they are separated according to their m/z ratio. The mass range of interest is scanned, causing separation of ions in space or time domains. Two mass analyzers, the magnetic sector and the quadrupole, are shown in Figure 7.7. The magnetic sector analyzer utilizes an electromagnet to separate ions in space according to the radius of their trajectories. The relationship of magnetic field strength to m/z is given by

$$m/z = \frac{B^2 r^2 e}{2\ V} \tag{7.1}$$

where B is the magnetic field strength, r is the radius of trajectory, e is the electron charge, z is the number of charges, and V is the accelerating voltage. In a typical magnetic sector analyzer, the magnetic field strength is varied, directing the ion beam across a narrow slit through which ions of increasing or decreasing m/z are selected. In this way a full-range mass spectrum is obtained. It is important that the magnet be scanned quickly enough to sample a chromatographic peak as it elutes. This was difficult with capillary GC in the past, but today faster scanning magnets can easily cover the range from 40 to 500 m/z in 0.5 s. This sampling rate (2 Hz) would yield 20 scans across a 10-s-wide chromatographic peak. The costs for these instruments are high when compared to those of other mass spectrometers; however, the higher mass resolution is generally considered to be an advantage.

The other common mass analyzer is the linear quadrupole. This mass analyzer consists of four cylindrical rods oriented in a square arrangement as shown in Figure 7.7. Radiofrequency (RF) and direct-current (DC) potentials are applied

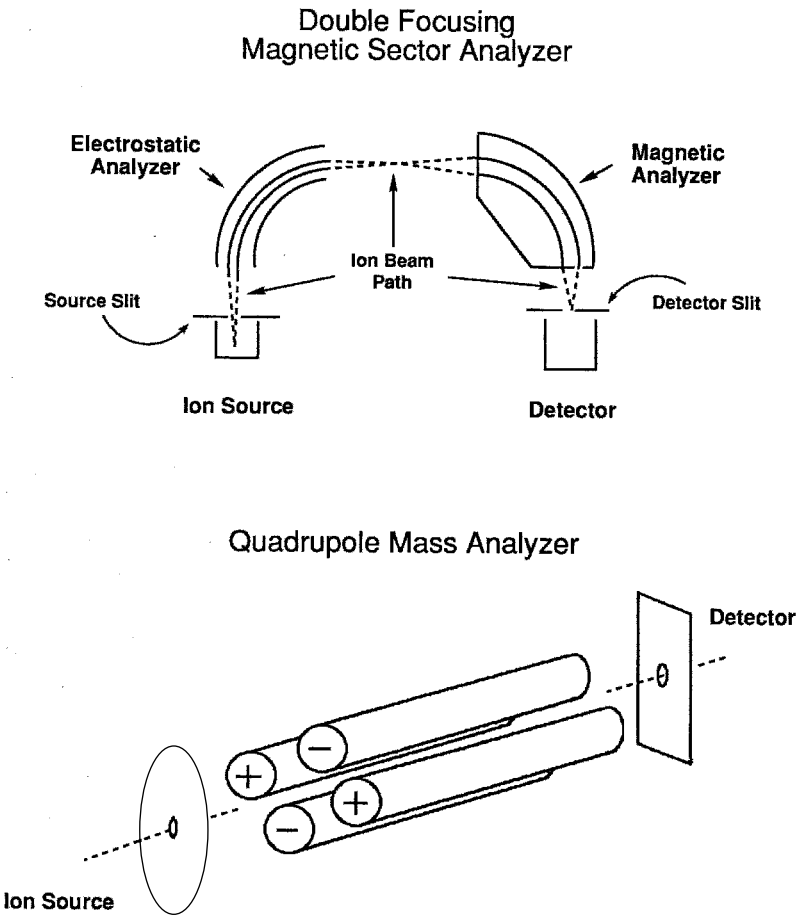

FIGURE 7.7 The two most popular mass analyzers. The magnetic sector instrument is normally configured in tandem with an electrostatic analyzer that narrows the kinetic energy spread of the ion beam. For this reason, it is called a double-focusing spectrometer.

to the rods enabling ions with a specific m/z to have a stable trajectory and pass through to the detector. By simultaneously increasing the RF and DC potentials, ions of increasing m/z will pass through the analyzer and be detected. Equations describing these ion trajectories are discussed elsewhere (37). Two advantages of the quadrupole are its fast scanning rate and lower cost. For these reasons, this is the analyzer most commonly used in GCMS.

Two other analyzers now commonly interfaced with GC are shown in Figure 7.8. These include the ion trap analyzer in which ions can be confined by electric and magnetic fields (38–40). Ion traps are essentially three-dimensional quadrupoles. Ions of a specific m/z value circulate in stable orbits within the analyzer. A relatively high pressure of helium (10^{-4} Torr) is used as a bath gas

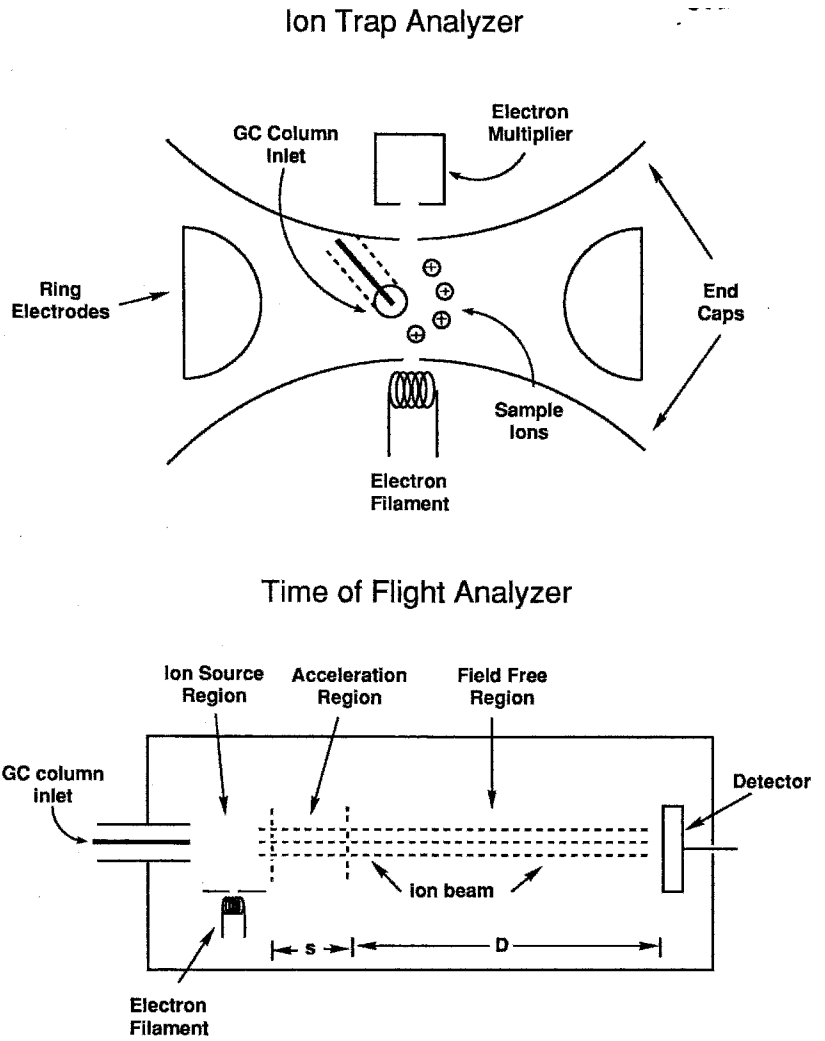

FIGURE 7.8 The ion trap and time-of-flight mass analyzers.

in order to stabilize the ion trajectories. As the RF frequency is increased, ions of lower m/z values are destabilized and pass into the detector. Ions are typically introduced from the ion source in a pulsed fashion and the RF frequency quickly scanned to produce a spectrum. These analyzers are quite inexpensive and very sensitive and are very popular as detectors for GCMS. A review of the ion trap literature from 1995 to 2001 has recently been published showing the wide variety of applications of this technology (41).

The time-of-flight (TOF) analyzers separate ions according to their migration time down a field-free region after acceleration. Since ions with equivalent kinetic

energy are produced, those of lower m/z arrive first followed in sequence by those of higher m/z:

$$m/z = 2Ese\left(\frac{t}{D}\right)^2 \tag{7.2}$$

In this equation, e is the electron charge, z is the number of charges, E is the magnitude of electrical field over which the ions are accelerated, s is the acceleration distance, t is the ion migration time, and D is the length of the field-free drift region (42–44). The use of TOF in GCMS has gained considerable interest because of scan speed and mass accuracy. Since the TOF mass filter is an integrating rather than a scanning detector, the acquisition rate is limited only by the ion pulse frequency and the spectrum storage speed. The current computer technology allows spectrum storage speed on the order of 500 spectra per second (45). These ultrafast GCMS experiments allow complex mixtures to be analyzed in a few seconds. In addition, with the introduction of electrostatic reflectron technology and the use of delayed extraction or time-lag focusing, sufficient mass resolution has been achieved to perform accurate mass determination at 5 ppm (46). These improvements have made GC/TOFMS an alternative to magnetic sector instruments for accurate mass measurements.

The degree of mass separation between adjacent ions (10% valley definition) is referred to as the *mass resolution* and is defined as $R = M/\Delta M$, where R is the resolution, M is the nominal mass of the ions, and ΔM is the difference in mass. All mass analyzers used in GCMS are capable of at least resolving unit mass to m/z 1000 for a resolution of 1000. This resolution is sufficient for the majority of applications. However it is sometimes necessary to accurately determine the mass of an unknown sample component to aid in its identification, by determination of its elemental composition. This is frequently done with a high-resolution analyzer such as the double-focusing magnetic sector instrument. With this instrument it is possible to differentiate species such as benzene (C_6H_6, MW = 78.0469 μ) and dimethyl sulfoxide (C_2H_6OS, MW = 78.0139 μ), which require a resolution of approximately 2400 to separate. Resolutions to 40,000 are possible with gas chromatography/Fourier transform mass spectrometry (GC/FTMS); however, the transient nature of chromatographic peaks and the rapid scanning makes the measurement more difficult (47,48). Table 7.1 compares some common features of mass spectrometers.

7.2.8 Detectors

Mass resolved ions travel from the analyzer to the ion detector. The detectors used in MS are required to have fast response and a large gain to convert the small ion currents generated into recordable signals.

The most popular detector, the electron multiplier, is shown in Figure 7.9. The ions collide with the first of a series of dynodes. The dynodes are operated at 1–3 kV, each one in the series maintained at a higher voltage. The effect is multiplication of the primary ion beam for a current gain of about 10^5. Frequently

TABLE 7.1 Common Features of Mass Spectrometers

Feature	Magnetic	Quadrupole	Ion Trap	TOF
Mass range in daltons (μ)	1–50,000	2–4000	10–2000	No limit
Acquisition	Full-scan SIM[a]	Full-scan SIM	Full-scan SIM	Full scan
Resolution	High 0.001 (m/z 1000)	Unit mass 1 (m/z 1000)	Unit mass 1 (m/z 1000)	High
Mass accuracy	Accurate (1 ppm)	Nominal	Nominal	Accurate (5 ppm)
Lower detection limit (g)	10^{-15}–10^{-14}	10^{-13}–10^{-12}	10^{-13}–10^{-12}	—
Linearity (order of magnitude)	3–4	4–5	3–4	
Scanning	Slow	Fast	Fast	Ultrafast

[a] Selected-ion monitoring.

Source: References 25 and 27.

a higher voltage (5–20 kV) conversion dynode is inserted before the multiplier to increase the ion beam energy prior to detection.

Another detector used in GCMS is the photomultiplier. These are similar in design to those used in optical spectroscopy. The ion beam collides with a phosphor-coated target, which converts the ions into photons that are subsequently amplified and detected. These detectors are typically operated at lower voltages (400–700 V) and last longer than conventional electron multipliers since they are sealed units that are not subjected to external contamination.

To enable detection of many masses simultaneously, a photographic plate had been used in the past in combination with magnetic sector instruments. Todays substitute, for multiple mass detection, is the multichannel array detector (Figure 7.9). A series of evenly spaced detectors are configured in a linear array. A selected range of ions is directed onto the array, allowing simultaneous recording of many masses. This enhances the overall sensitivity of measurement extending MS for applications such as trace analysis of unknown components. However, the specialized nature of this detector combined with its substantial cost has limited its use to date. GC/TOFMS with its higher acquisition rates has superceded the use of array detectors in most cases.

7.2.9 Scanning Techniques

The mass spectrometer is used to perform many tasks including, identification of unknowns, trace-level analysis, target compound quantification, and accurate mass measurements, to name a few. Each of these analyses has a number of specific instrumental requirements, including a preferred scanning mode.

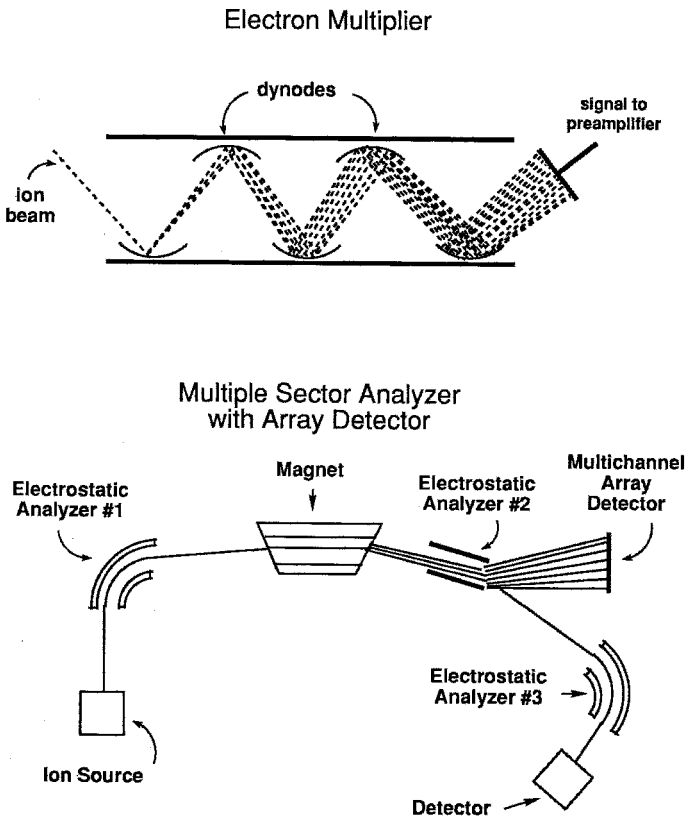

FIGURE 7.9 The electron multiplier and array detectors.

The most convenient method of mass scanning is full-mass-range scanning. In this scan mode, the spectrometer is scanned over a mass range covering all predicted molecular and fragment ions produced for a complex multiple component sample. For most GCMS of unknowns, this requires scanning the analyzer from about 50 to 600 μ. This lower mass (50 μ) is chosen to exclude the background ions produced from residual air, carrier gases, and CI reagent gases normally used. The upper limit is based on useful volatility of analytes. Many compounds above this molecular mass (600 μ), with the exception of specifically prepared volatile derivatives, have insufficient vapor pressure for analysis. In addition, for many spectrometers this represents the maximum practical mass range for capillary column use, since scanning speeds of 0.5 s per scan become more difficult beyond this range.

Another scanning mode frequently used is selected-ion monitoring (SIM). With this method the mass analyzer can be set to sample a single m/z value over the course of the chromatographic separation. By monitoring only a single m/z, the sensitivity is enhanced up to three orders of magnitude depending on mass

range, since the instrument does not spend time sampling undesired masses. This technique is useful for analyses such as quantification of target compounds, where the base peak of the analyte is normally chosen as the monitored m/z. It is also useful for analytes that are only partially resolved, since a unique ion can usually be chosen, which is not produced by the coeluting species. The computerized control possible with modern spectrometers allows the selected ion masses to be changed during the course of a separation, enabling optimization of analyses for all individual mixture components. SIM scanning epitomizes the selectivity and sensitivity possible with GCMS.

A variation of SIM involves multiple-ion monitoring (MIM). This scanning method allows selection of several discrete masses. These can be a molecular and/or fragment ion(s) of a single analyte or of different analytes. This technique can be used as a compromise between full-mass-range scanning for best qualitative information and single-mass SIM for increased sensitivity, by choosing several characteristic peaks from the desired components. Modern computers have significantly improved this process as specific mass ranges can be selected as the course of the separation proceeds. This allows, for example, shifting the scanned range to higher values as the column oven is heated over the course of a gradient separation since with many analog series, elution time, and/or temperature increases with molecular mass.

For optimal selectivity, particularly for quantitation with ion trap or triple quadrupole analyzers, MS/MS scanning techniques can be utilized in GCMS. With these techniques, instruments are operated to perform one of three basic experiments including product ion analysis, precursor ion analysis or neutral loss analysis. Ion traps are normally limited to product ion scans in which a particular ion of interest is isolated in the ion trap, subjected to collisional activation and its fragments are detected. Triple quadrupoles can additionally be operated to detect all precursors that generate a common fragment or the analyzer can be set to detect a specific neutral loss characteristic of an analyte functional group. All these methods add specificity to mass spectral detection.

7.2.10 Data Presentation

Going hand in hand with the scanning techniques described above, specific data presentation formats are used in GCMS. Some of these are summarized in Figure 7.10. The most common format used with full-mass-range scanning is the total-ion current (TIC) chromatogram (Figure 7.10a). This signal represents the summed ion current for the peaks detected in each mass spectral scan. Depending on response factors, this chromatogram frequently resembles the flame ionization detector trace. Usually, the mass spectral scan recorded at the maximum ion current for each chromatographic peak is presented as its characteristic spectrum. This is not usually a problem unless the scan rate is too slow relative to the peak width (less than 10 scans across the peak). Alternatively, spectra can be averaged to normalize variations in analyte concentration as each elutes into the ion source.

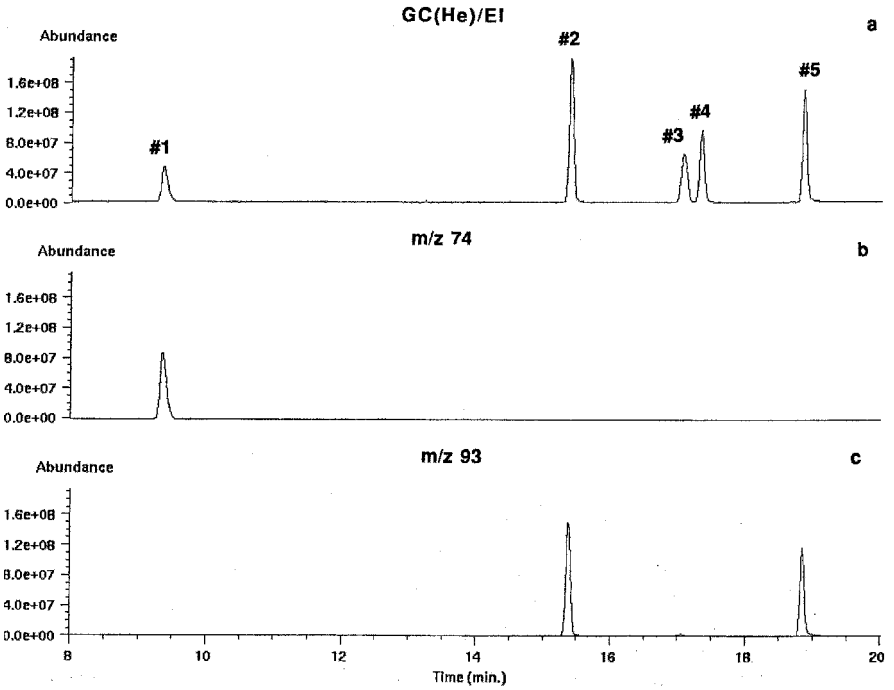

FIGURE 7.10 (a) GC/EIMS total-ion current (TIC) chromatogram of a five-component mixture of (1) (*N*-nitrosodimethylamine), (2) (Bis(2-chloroethyl)ether), (3) [bis(2-chloroisopropyl)ether], (4) (*N*-nitrosodi-*n*-propylamine), and (5) [bis(2-chloroethoxy)methane]. GC/EIMS obtained under the following conditions: GC conditions—column DB-1 (30 m × 0.320 mm); film thickness 5.00 μm; carrier gas helium at 25 cm/s; oven program 45°C for 3 min. then 10°C/min to 300°C for 12 min; injection port 265°C; sample 1 μL at 2000 μg/μL; solvent methylene chloride; samples were injected in the splitless mode (0.75 min load). MS conditions—mass range 50–500 μ; electron energy 70 eV; repeller 7.0 V; GCMS interface temperature 250°C; ion source temperature 200°C. (b) Mass chromatogram of *m/z* 74. (c) Mass chromatogram of *m/z* 93. (Masucci and Caldwell, unpublished data.)

In cases in which target compound analysis is being performed, chromatograms can be generated in which a single *m/z* is profiled. This is usually an intense or structurally characteristic peak that will identify the analyte of interest. These traces are often referred to as *selected ion* or *mass chromatograms*, and the method of data retrieval is called selected ion extraction. It differs from SIM in that the ion is selected postacquisition from the full-mass-range data. This technique is also valuable in cases where two or more components are not chromatographically resolved. By selecting ions characteristic of each of these analytes, and plotting these mass chromatograms, a broad peak can be deconvoluted into its individual components, and peak purity can be determined. Mass chromatograms are shown in Figure 7.10b,c. To complement multiple-ion SIM,

multiple-ion extraction can also be performed to differentiate components that exhibit common intense peaks, but different minor peaks, such as in the case of a hydrocarbon or other chemical series.

7.2.11 Background Artifacts

There are several sources of contamination in GCMS. As mentioned above, many liquid phases used in GC columns have appreciable vapor pressure and thus can bleed into the ion source along with the effluent. Vacuum pump oil also has significant vapor pressure and can bleed into the ion source. Other very common contaminants include phthalates such as dioctyl and di-*n*-butyl phthalate, which are used to stabilize plastic or rubber seals. Organic solvents stored in plastic bottles or passed through plastic tubing can be contaminated with these plasticizers. An intense peak at m/z 149, which corresponds to the phthalic anhydride cation, will be observed for phthalates (Figure 7.15). These contaminants and others, such as air and water, make up the background of a GCMS experiment. Spectra of some of these are shown in Figures 7.11 and 7.12.

For a mass spectrum of a particular analyte, the spectrum is a mixture of the background and the analyte. If a representative mass spectrum of the background

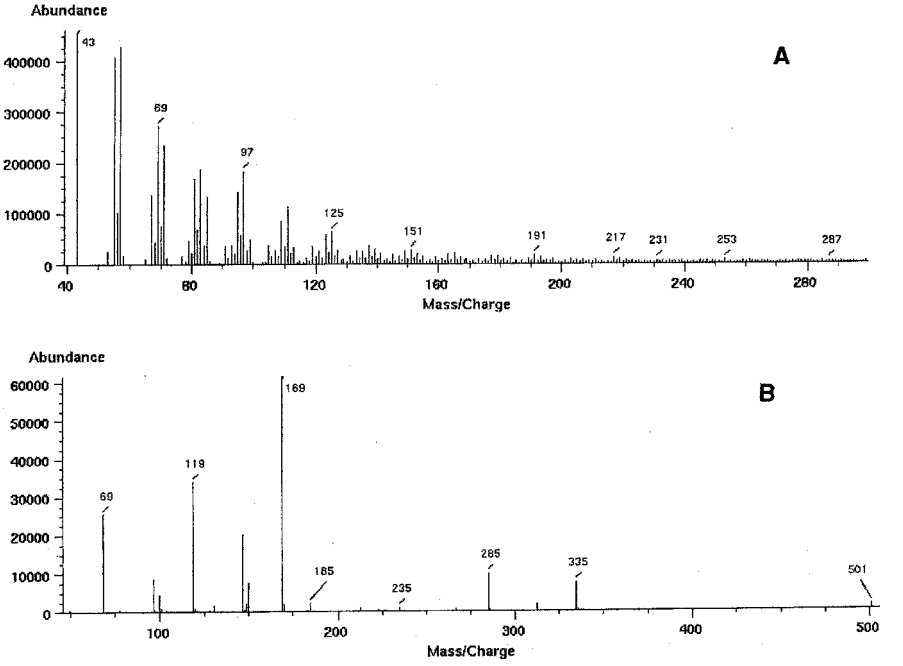

FIGURE 7.11 Electron ionization mass spectra of vacuum pump oil: (a) hydrocarbon-based (Inland 19); (b) fluorocarbon-based (Fomblin) (Masucci and Caldwell, unpublished data).

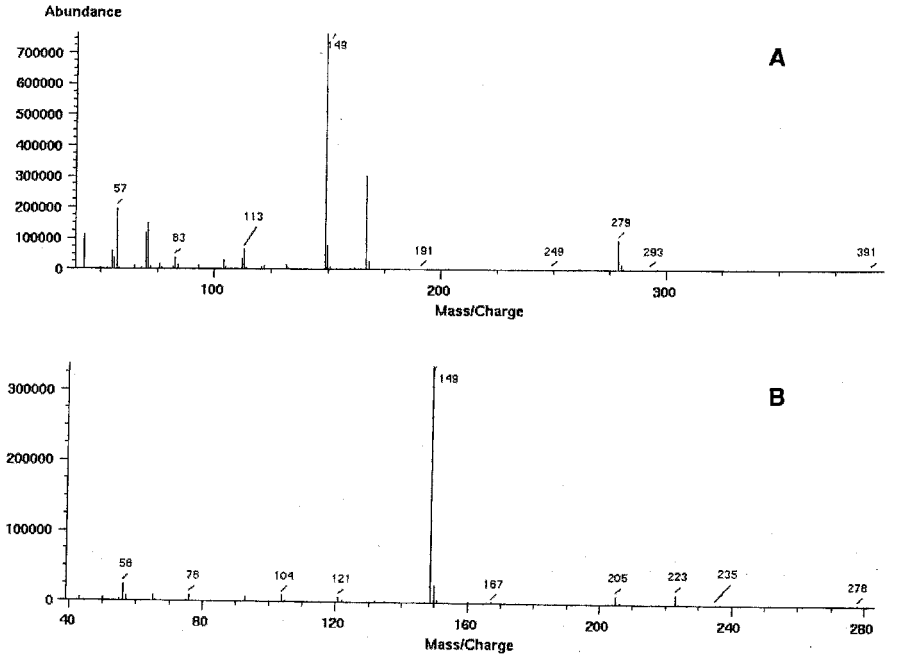

FIGURE 7.12 Electron ionization mass spectra of phthalates: (a) dioctyl phthalate; (b) di-*n*-butyl phthalate (Masucci and Caldwell, unpublished data).

can be obtained, it is desirable to subtract the background spectrum from the analyte spectrum. The resulting subtracted mass spectrum of the analyte is then of the "pure" substance.

7.3 GAS CHROMATOGRAPHY/ELECTRON IONIZATION MASS SPECTROMETRY

7.3.1 Electron Ionization

There are many general reviews of electron ionization (EI) mass spectrometry (49–53). Key factors that affect the technique are described here.

The neutral molecules [M] that elute from the gas chromatographic column must be charged positively or negatively in order to manipulate them in mass analyzers. As these neutral molecules randomly diffuse throughout the ion source, they are bombarded with electrons at typically 70 eV of energy. If an electron with sufficient energy collides and knocks out of orbit one of the neutral molecule's electrons, a radical cation $[M]^{+\bullet}$ is formed (Reaction 7.3). This $[M]^{+\bullet}$ radical cation is referred to as the molecular ion. If the molecule captures the electron, a radical anion $[M]^{-\bullet}$ is formed (Reaction 7.4). It should be remembered that most of bombarding electrons are elastically scattered and do not form

ions. Therefore, only a few hundredths of a percent of the sample molecules are ionized to molecular ions under these conditions with the bulk of the sample molecules being removed by the vacuum pumps. Negative ions are 10^4 less abundant than positive ions at 70 eV. One might think, with such a low concentration of ions being formed, that the EI technique would be insensitive. On the contrary, only a few femtomoles of sample are required to be detected with modern spectrometers.

$$e^- + [M] \rightarrow [M]^{+\bullet} + 2e^- \tag{7.3}$$

$$e^- + [M] \rightarrow [M]^{-\bullet} \tag{7.4}$$

The electrons that are emitted from the filament in the ion source have a distribution of energies from 0 eV to greater than 20 eV (1 eV = 96.3 kJ/mol). The ions generated under these conditions have a distribution of internal energies ranging from 2 to 6 eV. Thus, some of the radical cations $[M]^{+\bullet}$ and the radical anions $[M]^{-\bullet}$ have considerable excess energy and some have very little. It is this excess energy that makes the radical cations and anions unstable and provides the source of energy that could fragment almost any single bond contained in the molecule. Organic molecules have bond energies approximately in the range 2–6 eV (193 to 578 kJ mol^{-1}). The molecular ion with its excess energy undergoes a unimolecular decomposition reaction where one fragment retains the positive charge $[F_1]^+$ and the other is a neutral radical $[N_1]^\bullet$ (Reaction 7.5). The molecular ion can fragment via single-bond cleavages to many different fragment types ($[F_1]^+$, $[F_2]^+$, $[F_3]^+$, etc.). In some cases, the molecular ion can rearrange such that two bonds are cleaved and two are formed to produce a radical cation and neutral molecule (Reaction 7.6). Since all chemical bonds are not of equal strength, the fragmentation process is not random and structurally characteristic fragments are produced. The excess energy in the $[M]^{-\bullet}$ ion causes it to convert back to a neutral molecule (Reaction 7.7), which accounts for its low concentration in the ion source. Reactions 7.3–7.6 all occur in the ion source simultaneously:

$$[M]^{+\bullet} \rightarrow [F_1]^+ + [N_1]^\bullet \tag{7.5}$$

$$[M]^{+\bullet} \rightarrow [R]^{+\bullet} + [N] \tag{7.6}$$

$$[M]^{-\bullet} \rightarrow [M] + e^- \tag{7.7}$$

The thermochemical relationship for Reaction 7.5 can be expressed as the ionization energy (IE) of the radical fragment [F$^\bullet$] less the IE of the neutral sample molecule [M] plus the bond dissociation energy [D] of the F–N bond [i.e., ΔH (Reaction 7.5) = IE(F$^\bullet$) − IE(M) + D(F–N)]. The ionization energy is the amount of energy required to remove an electron from the species under consideration. The bond dissociation energy is the amount of energy required to produce a homolytic bond cleavage. Several sources of thermochemical data can be used to calculate the exothermicity of Reaction 7.5 (54,55). The importance of positive

charge stabilization and bond strength is reflected in ΔH (Reaction 7.5). Thus, a comparison of two competitive fragmentation reactions from the same sample molecule, the difference in the IEs and the difference in the bond dissociations control the abundance of the pathways. In other words, the fragment that can best stabilize the positive charge will predominate the EI spectrum.

The molecular ion is observed in the mass spectrum in varying abundance. When the molecular ion retains a large amount of excess energy, the $[M]^{+\bullet}$ cation may totally fragment and not be observed in the mass spectrum. A lower voltage on the filament such as 10–15 eV may increase the abundance of the molecular ion. Fragmentation of the molecular ion decreases since less excess energy is transferred to the $[M]^{+\bullet}$. Thus, lower electron energies drastically alter the overall spectrum as well as maximize molecular ion production. However, the total-ion production decreases, causing lower sensitivity. Electron energy variations in the range of 55–85 eV are rather insignificant in the overall appearance of the GCEI technique. For this reason, EI mass spectra are highly reproducible from various manufactured instruments.

7.3.2 Qualitative Methods: Structure Elucidation

The EI fragments are pieces of the original molecule and provide a way to determine its structure. McLafferty (52,56) has created a step-by-step procedure for interpreting an unknown mass spectrum. While it is not the intention to repeat the entire procedure here, useful key steps in this process are reviewed below:

1. The sample's history is probably the most valuable piece of information to have when determining an unknown structure. Time should be taken to obtain as much information as possible.
2. A mass spectrum of the unknown should be obtained free of any artifacts (see Section 7.2.10).
3. The molecular ion and thus, the molecular weight of the sample molecule, should be determined. Since EI mass spectra frequently do not contain a molecular ion (see Figure 7.4), an alternate ionization technique (see Sections 7.4 and 7.5) or lowering electron energy (see Section 7.3.1) should be attempted to establish the molecular weight.
4. The recognition of natural abundance of isotopes in a mass spectrum can provide elemental composition of the peak at an m/z value. Table 7.2 lists the natural abundances of the commonly encountered atoms. Note that fluorine, phosphorus, and iodine (not listed in table) have no stable heavy isotopes. The presence of stable isotopes in the sample molecule results in the mass spectrum containing peaks with multiplicity. Consider the mass spectrum of acetaminophen in Figure 7.2, where the peak m/z 151 is the molecular ion that has an elemental composition of $C_8H_9NO_2$. The peak at m/z 152 is due to the naturally occurring ^{13}C isotope of acetaminophen, $^{12}C_7^{13}CH_9NO_2$, and has a relative intensity of 0.011 multiplied by the number of carbon atoms (Table 7.2). Compounds containing an odd number of

TABLE 7.2 Natural Abundance of Stable Heavy Isotopes

Name	Element	Abundance	Isotope	Abundance	Isotope	Abundance
Hydrogen	^{1}H	99.99	^{2}H (or D)	0.01	—	—
Carbon	^{12}C	98.9	^{13}C	1.1	—	—
Nitrogen	^{14}N	99.6	^{15}N	0.4	—	—
Oxygen	^{16}O	99.76	^{17}O	0.04	^{18}O	0.20
Silicon	^{28}Si	92.9	^{29}Si	4.7	^{30}Si	3.1
Sulfur	^{32}S	95.02	^{33}S	0.76	^{34}S	4.22
Chlorine	^{35}Cl	75.77	—	—	^{37}Cl	24.23
Bromine	^{79}Br	50.5	—	—	^{81}Br	49.5

Source: References 52 and 56.

nitrogen atoms, such as acetaminophen, will have an odd nominal molecular mass. Those compounds with an even number of nitrogens will have an even nominal mass. This is called the *nitrogen rule* and is valid because nitrogen has an even atomic mass and an odd valence. The atoms C, H, O, S, Si, and the halogens either have an even atomic mass and a even valence or an odd atomic mass and an odd valence. Isotopes of Cl and Br are easily recognized in a mass spectrum. In Figure 7.4b is shown the mass spectrum of methylene chloride (CH_2Cl_2). The peak at m/z 84 is the molecular ion, which has an elemental composition of $CH_2^{35}Cl_2$; the peak at m/z 86 has an elemental composition of $CH_2^{35}Cl^{37}Cl$; the peak at m/z 88 has an elemental composition of $CH_2^{37}Cl_2$. The base peak at m/z 49 is simply the [M–Cl] cation. Note that the ratio of the abundances of the peaks at m/z 49 (100%) and 51 (33%) is approximately 3–1. This ratio is consistent with this fragment containing one chlorine atom (Table 7.2). Note that the ratio of the abundances of the peaks at m/z 84 (65%), 86 (42%), and 88 (7%) is approximately 9–6–1. This ratio is consistent with this fragment containing two chlorine atoms (27).

5. If the molecular ion can be recognized, a strategy for interpretation is to calculate losses from the molecular ion that might account for the fragments. Some common losses are listed in Table 7.3. Again note that the base peak at m/z 49 in Figure 7.4b is simply the loss of a Cl atom from the molecular ion at m/z 84.
6. Try to recognize mass series of fragments that are unique to a particular functional group. For example, if a series of peaks at m/z 43, 57, 71, 85, 99, 113, and 127 (note the 14 μ mass difference) were observed, it can be concluded that the unknown sample molecule contains a long chain saturated hydrocarbon tail (Figure 7.11).

Finally, all the history, fragmentation, and isotope information is combined in the postulation of possible molecular structures. Detailed mechanisms have been published for organic compounds containing a wide variety of functional groups to help in this process (51,52,56).

TABLE 7.3 Common Losses from the Molecular Ion

Molecular Ion	Species	Molecular Ion	Species
M-15	CH_3	M-41	C_3H_5
M-16	O, NH_2	M-42	C_3H_6, CH_2CO
M-17	OH, NH_3	M-43	C_3H_7, CH_3CO
M-18	H_2O	M-44	C_3H_8, CO_2
M-19	F	M-45	CO_2H, OC_2H_5
M-20	HF	M-46	CH_3CH_2OH
M-27	HCN	M-55	C_4H_7
M-28	CO, C_2H_4	M-57	C_4H_9
M-29	CHO, C_2H_5	M-58	C_4H_{10},$(CH_3)_2CO$
M-30	CH_2O, C_2H_6	M-60	CH_3COOH
M-31	OCH_3	M-73	$(CH_3)_3Si$
M-32	S, $HOCH_3$	M-79	Br
M-34	H_2S	M-89	$(CH_3)_3SiOH$
M-35	Cl	M-127	I

Online computer comparison of the mass spectrum of an unknown analyte against a reference mass spectral library was developed to aid the interpreter (56). Since the early 1980s, there has been a steady growth in the understanding of how to use computers to interpret mass spectral information. It is not surprising that this development of library search routines has been strongly influenced by computer technology. For example, the Wiley mass spectral library contains approximately 310,000 mass spectra, while the NIST (National Institute of Standards and Technology) library contains about 130,000 spectra. Both libraries are commercially available and can be implemented from the GCMS workstation. Several successful algorithms for comparing the mass spectrum of an unknown against a library of known compounds have been developed and are available on most commercial mass spectrometer data systems. The most widely used algorithm is the probability-based matching (PBM) software. This software can search 220,000 reference mass spectra in approximately 3 s (56). The PBM uses statistical information gathered from mass spectral databases to assign "uniqueness" to mass spectral peaks. For example, a fragment at m/z 57 with an abundance of 100% occurs quite frequently in mass spectra. Since this peak has a high probability of occurrence, it is given a low uniqueness value. A fragment at m/z 570 with an abundance of 100% would be given a high uniqueness value since this combination of mass and abundance occurs infrequently in mass spectra. Comparison of the mass spectrum of an unknown sample molecule against a reference mass spectral library is called *forward searching*. Comparison of the reference library against an unknown is called *reverse searching*. All mass spectra are a mixture of the analyte and the background. In a forward search these background peaks are included in the search; however, the reverse searching procedure ignores peaks in the unknown that are not in the reference spectrum. Reverse searching typically gives superior search results.

7.3.3 Quantitative Methods

Since GCMS has excellent selectivity of detection and good limits of detection for many compounds, it is routinely used for quantitative analyses. It has been applied to biological fluids, such as blood plasma and urine (57), environmental samples (58), petroleum samples (59), agricultural problems (60), law enforcement (61), clinical studies (1), and polymer chemistry (62). Almost all quantitative applications of GCEI rely on stable isotope dilution techniques or internal standards similar in structure to the compound of interest and selected-ion monitoring (SIM) recording methods. Stable isotopically labeled analogs of compounds serve as ideal internal standards and are added to the sample matrix to correct for losses during sample preparation, and GCEI analyses. Multiply deuterated or ^{13}C-labeled compounds with a mass difference of at least 3 μ from the unlabeled compound are preferred since they preclude overlap in simultaneous recording of selected ion pairs. Complex biological matrices, such as urine or plasma, are extracted into organic solvents and purified by chromatographic methods. Because of certain analyte functional groups (e.g., ROH and RCO_2H), these samples may have poor gas chromatographic properties leading to tailing peaks and/or unfavorable mass spectrometric properties leading to insensitive detection. These samples are generally derivatized to improve gas chromatographic properties (peak symmetry, volatility, thermal stability) by replacing any active hydrogen atom (–OH, $–NH_2$, –NHR, –SH) in a molecule with a trimethylsilyl group. A calibration curve is typically constructed by analyzing standard solutions containing varying amounts of the sample of interest and a fixed amount of internal standard. Calibration curves are typically linear with a correlation coefficient >0.99. Measuring the precision and accuracy between days and within day tests the ruggedness of the method.

The development of an analytical assay for the determination of drugs in biological fluids is always a challenging problem. An example, from the literature, of a GCEI method for the quantitative analysis of a drug from plasma is used here to illustrate important points (57). Biperiden is an anticholinergic drug that has been used clinically for central nervous system degenerative disorders. The pharmacokinetic parameters in guinea pigs following intramuscular administration of a 0.5 mg/kg dose of biperiden were determined. The experiment used 290 μL of plasma and a simple hexane extraction/clean up procedure to prepare the samples for GC/EI quantitative analysis. It was not necessary to derivatize these samples since the gas chromatographic properties were acceptable. Trihexylphenidyl a compound similar in structure to biperiden was used as the internal standard. The mass spectrometer was operated using selected ion monitoring for two cations (m/z 98 and 218) characteristic for both biperiden and trihexylphenidyl. The calibration curve of biperiden was linear over the range 1.9–250 ng/mL with correlation coefficients between 0.9984 and 0.9999. For between-day and for within-day precision, the coefficient of variations were similar (1–5%). The accuracy as expressed by percentage error ranged from -3 to 5% for between-day and for within-day precision. The method described by these

authors (57) produced the required precision, accuracy, and sensitivity to assay biperiden at the doses utilized for their pharmacokinetic studies.

7.3.4 Negative-Electron Ionization

Negative ions generated from electron ionization (EI) generally give poor sensitivity because negative-ion production requires electrons of much lower energy (~0 eV) to facilitate electron capture and ion pair production. Halide ions (F^-, Cl^-, Br^-, and I^-) are typically observed as low-level background anions in this mode. However, negative-ion chemical ionization (NICI) can generate negative ion abundances comparable to EI. A description of NICI is given in Section 7.5.

7.4 GAS CHROMATOGRAPHY/POSITIVE-ION CHEMICAL IONIZATION MASS SPECTROMETRY

7.4.1 Advantages of Positive-Ion Chemical Ionization

An extremely useful alternative to electron ionization mass spectrometry (EIMS) is positive-ion chemical ionization (PICI) mass spectrometry. The PICI technique was developed by Field and co-workers (63–65), and general reviews of this technique by Munson (66–68), Harrison (69), and Bartmess (70) have appeared. Briefly, the difference between EI and PICI can be understood simply by considering the amount of energy deposited into the sample molecules during the ionization process. In EI, sample molecules are ionized to radical cations by electrons. The excess energy deposited in these radical cations can range from near 0 to 70 eV and can cause extensive fragmentation. In PICI, sample molecules are ionized to cations by other cations and the excess energy deposited in these cations depends on the thermochemistry of the cation/molecule reaction. The energy range of these cation/molecule reactions is much narrower (near 0 to ~20 eV), which results in much less fragmentation.

As discussed above, sample molecules [M] ionized by electrons (Reaction 7.8) retain some fraction of the 70-eV energy beam internally. Depending on the amount of excess internal energy retained by the $[M]^{+\bullet}$ cations and the number of labile bonds, these cations generally further dissociate to yield fragment cations and neutral radicals (Reaction 7.9). Remember that the amount of energy required to break a bond in a typical organic molecule ranges only from ~2–6 eV (193–578 kJ/mol). In some cases the amount of internal energy retained by the $[M]^{+\bullet}$ cation is so great that the $[M]^{+\bullet}$ cation fragments completely. Thus, the $[M]^{+\bullet}$ cation is sometimes not observed in the EI spectrum. The lack of a molecular cation can greatly complicate the identification of unknowns and is a serious disadvantage of the EI technique:

$$e^- + [M] \rightarrow [M]^{+\bullet} + 2e^- \tag{7.8}$$

$$[M]^{+\bullet} \rightarrow [F_1]^+ + [F_2]^+ + [N]^\bullet \tag{7.9}$$

The PICI technique requires that a gaseous mixture consisting of a reagent gas [R] (e.g., methane, isobutane, or ammonia) and the sample molecule [M] of interest is present in the ion source in a molar ratio of approximately 1000−1. Since the reagent gas is in much larger excess to the sample molecules, virtually all primary cations are produced by direct electron ionization of the reagent gas (Reaction 7.10). The sample molecules are not ionized to any extent by the direct electron beam. These primary cations (C_p^+) will further react with the bulk reagent gas to produce a set of secondary cations $[C]_n^+$ that are unique to the reagent gas and are at a relatively steady-state concentration (Reaction 7.11). The number of secondary cations generated will vary from reagent gas to reagent gas; however, for the common gases there are generally not more than three major cations (69). At some point in time these secondary cations collide with [M] and a cation−molecule reaction occurs. Sample molecules ionized by these secondary cations typically produce protonated molecular cations $[M+H]^+$ (Reaction 7.12), hydride abstraction cations $[M-H]^+$ (Reaction 7.13), charge exchange cations $[M]^{+\bullet}$ (Reaction 7.14), and/or cluster adduct cations $[C+M]^+$ (Reaction 7.15). The abundances of these cations are controlled by cation/molecule reactions and ultimately depend on the specific reagent gas and its pressure and source temperature. More details on how these cations are created in PICI will be discussed in Sections 7.4.3–7.4.7. The $[M+H]^+$, $[M-H]^+$, $[M]^{+\bullet}$, or $[M+C]^+$ cations that are produced via the PICI technique are usually much less energetic than those formed in the EI process, and result in less fragmentation. The fragments produced by PICI are sometimes a different set of fragments than those produced by EI for the same molecule. The fragmentation of $[M+H]^+$, $[M-H]^+$, and $[M+C]^+$ cations usually involve the elimination of an even-electron neutral (a stable molecule) to form an even-electron fragment cation. It should also be noted that $[M+H]^+$, $[M-H]^+$, and $[M+C]^+$ cations are even-electron cations, which, in general, are more stable than the odd-electron cations produced in EI. Therefore, in many cases, the $[M+H]^+$, $[M-H]^+$, or $[M+C]^+$ cation is observed in relatively high abundance with a limited number of fragment cations.

$$e^- + [R] \rightarrow C_p^+ + 2e^- \tag{7.10}$$

$$C_p^+ + [R] \rightarrow [C]_1^+ + [C]_2^+ + \cdots + [C]_n^+ \tag{7.11}$$

$$C^+ + [M] \rightarrow [M+H]^+ + [C-H] \tag{7.12}$$

$$C^+ + [M] \rightarrow [M-H]^+ + [C+H] \tag{7.13}$$

$$C^{+\bullet} + [M] \rightarrow [M]^{+\bullet} + C \tag{7.14}$$

$$C^+ + [M] \rightarrow [M+C]^+ \tag{7.15}$$

In Figure 7.13 examples of EI, methane PICI and an interesting cation/molecule reaction called self-PICI are shown. Figure 7.13a is the 70 eV EI spectrum of an aromatic compound in which the $[M]^{+\bullet}$ cation (m/z 429) is not observed. For this molecule, the amount of internal energy retained by the $[M]^{+\bullet}$ cation, after electron ionization, was so great that the $[M]^{+\bullet}$ cation totally fragmented

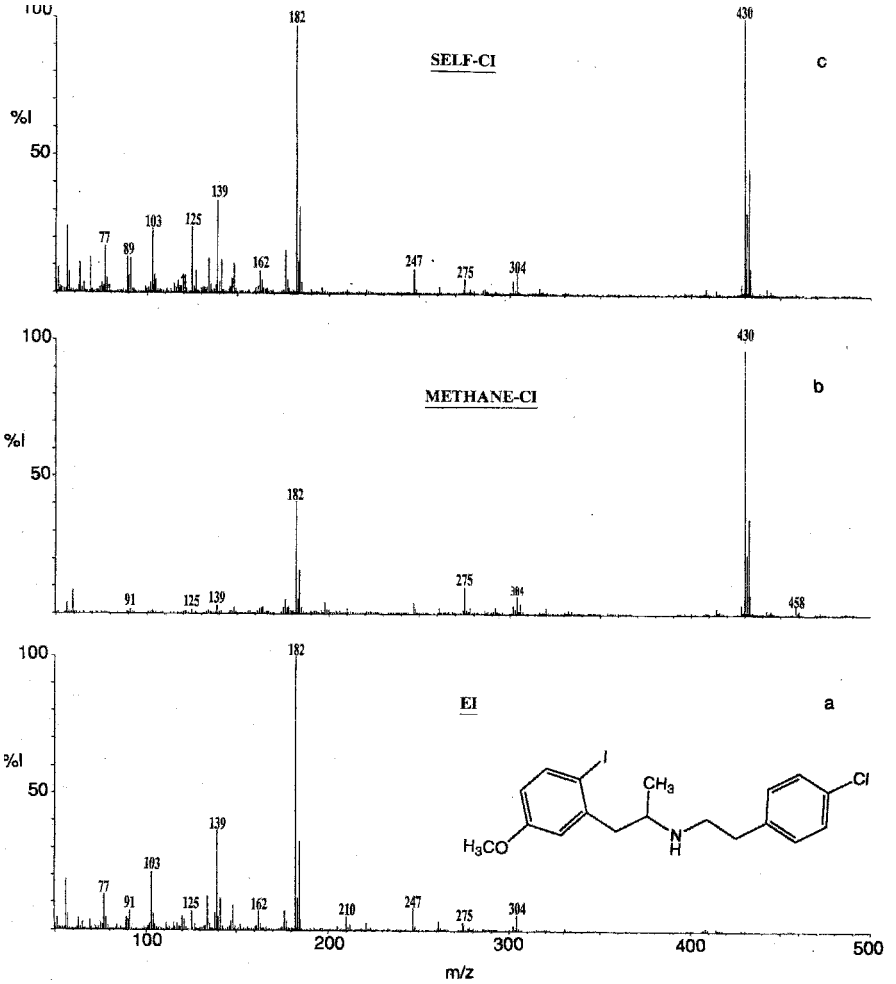

FIGURE 7.13 (a) EI spectrum of [2-(4-chlorophenyl)ethyl]-[2-(2-iodo-5-methoxyphenyl)-1-methylethyl]-amine (I) obtained under the following conditions: mass range 50 to 500 Daltons; electron energy 70 eV; ion source temperature 200°C; ion exit slit 1.5 × 7.4 mm. (b) PICI spectrum of (I) obtained under the following conditions: mass range 50–500 μ; electron energy 200 eV; ion source temperature 200°C; reagent gas methane ~0.1–0.2 Torr; ion exit slit 0.1 × 7.4 mm. (c) Self-PICI spectrum of (I) obtained under the following conditions: mass range 50–500 μ; electron energy 70 eV; ion source temperature 200°C; ion exit slit 0.7 × 7.4 mm. (Masucci and Caldwell, unpublished data.).

(Reactions 7.8 and 7.9). Figure 7.13b is the methane PICI spectrum of the same compound in which the $[M+H]^+$ cation (m/z 430) is the base peak (Reaction 7.12). Note there is less fragmentation in PICI as compared to EI. Figure 7.13c is an example of a phenomenon called self-PICI, which can show up in spectra if the pressure of the reagent gas is low and the pressure of the sample molecules

is high. That is, when the reagent gas is accidentally turned off and a sample molecule at high concentration (*ca.* 0.1 Torr) is introduced into a CI source, a mixed EI/CI spectrum is obtained. It is easy to see peaks from both the EI and PICI ionization processes in Figure 7.13c. Reactions 7.16 generalizes the cation/molecule reaction that produces the self-PICI peak.

$$[M]^{+\bullet} + [M] \rightarrow [M + H]^+ + [M - H]^{\bullet} \tag{7.16}$$

$$[M + H]^+ \rightarrow [M + H - X]^+ + [X] \tag{7.17}$$

$$[M + H]^+ + [M] \rightarrow [2\ M + H]^+ \tag{7.18}$$

Note that the $[M+H]^+$ cation is formed by transfer of a proton from $[M]^{+\bullet}$ to [M] or by transfer of a hydrogen atom from [M] to $[M]^{+\bullet}$. The $[M]^{+\bullet}$ (Reaction 7.9) and $[M+H]^+$ (Reaction 7.17) cations can both fragment. The [X] species in Reaction 7.17 is a stable neutral. If the analyte pressure is sufficiently high, dimers ($[2M+H]^+$) are also produced (Reaction 7.18). Self-PICI has been noted by several investigators (71–74).

7.4.2 Kinetic and Thermodynamic Considerations

To utilize GCCI, in general, an understanding of gas phase kinetics and thermodynamics is useful. Issues such as "what is the best reagent gas" or "what parameters affect CI and why" can best be understood from these data. Examination of rate constants for typical PICI reactions precedes the description of cation/molecule thermochemistry. Knowledge of thermochemistry can predict and/or rationalize many properties of chemical ionization and, thereby, reduce the effort of trial-and-error searching for optimum analytical conditions.

Bimolecular reactions, such as those in Reactions 7.12–7.14, have second-order rate constants (k) typically on the order of $1–4 \times 10^{-9}$ cm^3 molecule^{-1} s^{-1}(69). The number of collisions (Z; collisions s^{-1}) that occur between cations and sample molecules in the CI source can be estimated by multiplying the rate constant times the density of molecules in the source (N; molecule cm^{-3}). At a pressure of 0.5 Torr and a temperature of 473 K, the density (N) is approximately 10^{16} molecules/cm^3 and therefore, $Z = 1–4 \times 10^7$ collisions s^{-1}. The residence time (t) of most cations in a typical CI source is on the order of 10^{-5} s. More details on the parameters that effect the residence time of cations are given in Section 7.4.3. The number of collisions a cation undergoes is approximately 100–400 collisions ($Z \times t$). This range of collisions permits equilibria to be sufficiently established in order to assume a Boltzman distribution of internal energy of the cations. The clustering Reactions 7.15 have rate constants typically on the order of 10^{-27} cm^6 molecule^{-2} s^{-1}. Note that this is a third-order rate constant since it depends on the total pressure of the CI source. When a cation and a neutral molecule collide to form a complex $[M+C]^+$, it is initially in an excited state and must be stabilized by collisions with the reagent gas for observation. In the absence of such stabilization, the complex

decomposes. Assuming that the density N is approximately 10^{16} molecule cm^{-3} again, an effective second-order of 10^{-11} cm^3 $molecule^{-1}$ s^{-1} can be defined by multiplying N times the third-order rate constant. While this effective bimolecular rate constant is considerably smaller than the bimolecular rate constant quoted above, Reaction 7.15 is important in PICI for polar compounds and polar reagent gases capable of hydrogen bonding. While the values estimated above will vary somewhat from instrument to instrument, they will be of this order of magnitude. These results suggest that the kinetics are fast enough so that thermodynamic data can be used to examine the energetics that are pertinent to PICI.

The Gibbs free energy (ΔG°) of Reactions 7.12–7.15 is an important way of relating structure and reactivity. That is the net enthalpy (ΔH°) and entropy (ΔS°) changes that occur on the formation of new bonds and the breaking of old ones ($\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$). For the proton transfer Reaction 7.12, for example, a large positive ΔG° means that it will not take place. If ΔG° is large and negative, the proton transfer Reaction 7.12 will occur. The literature contains a great deal of thermochemistry that can be used to calculate the energetics of Reactions 7.12–7.15 (75).

Another way of examining the thermochemistry of Reaction 7.12, for example, is to consider the individual proton affinities (PAs) of the reagent gas [C–H] and the sample molecule [M]. The proton affinity (i.e., gas phase basicity) is generalized by Reactions 7.19 and 7.20:

$$[\mathrm{M}] + [\mathrm{H}]^+ \rightarrow [\mathrm{M} + \mathrm{H}]^+ \tag{7.19}$$

$$\mathrm{C}^+ \leftarrow [\mathrm{H}]^+ + [\mathrm{C} - \mathrm{H}] \tag{7.20}$$

As can be seen, the addition of Reactions 7.19 and 7.20 is simply Reaction 7.12; thus the energy for the transfer of a proton from the reagent cation to the sample molecule can be calculated by comparing the proton affinities of the reagent gas to the sample molecule [ΔH (Reaction 7.12) = PA (reagent gas) − PA (sample)]. If the sample molecule has a greater proton affinity than does the reagent gas, then the CI reaction can take place. The fundamental concept of proton affinity (basicity) is well defined within organic chemistry. The variation of proton affinity with structure has been examined by Taft (76) and Aue and Bowers (77). Useful relationships are found that allow unknown proton affinities to be estimated. For example, typical nitrogen-containing species have proton affinities in the range 854–1005 kJ/mol, sulfur-containing species are in the range 829–875 kJ/mol, and oxygen-containing species are in the range 754–853 kJ/mol. Table 7.4 lists the proton affinities of several possible reagent gases while Table 7.5 lists the proton affinities of several small organic molecules with various functional groups (69,77). The proton affinities of the reagent gas increase on proceeding down the table. By choosing the proper reagent gas, the PICI techniques can selectively protonate molecules. As an example, if the reagent gas were methane and the sample molecule were toluene [ΔH (Reaction 7.12) = 550 − 794 = −244 kJ/mol], the reaction would be exothermic and

TABLE 7.4 Examples of Proton Affinities (kJ/mol) and Ionization Energies (eV) of Reagent Gases

Species (B)	PA(B)[a]	IE(B)[b]	Cation Formed
He	178	24.6	HeH^+
H_2	423	15.4	H_3^+
CH_4	550	12.5	CH_5^+
C_2H_4	680	10.5	$C_2H_5^+$
H_2O	697	12.6	H_3O^+
H_2S	712	10.5	H_3S^+
CH_3OH	761	10.9	$CH_3OH_2^+$
i-C_4H_{10}	823	10.6	t-$C_4H_9^+$
NH_3	854	10.2	NH_4^+
CH_3NH_2	896	9.0	$CH_3NH_3^+$
Ar	—	15.8	
N_2		15.6	
O_2		12.1	
NO		9.3	

[a] Data from Reference 75.
[b] Data from Reference 69.

TABLE 7.5 Examples of Proton Affinities (kJ/mol), Hydride Affinities (kJ/mol), and Recombination Energies (eV)

Species (B)	PA(B)[a]	HIA(BH^+)[b]	RE(BH^+)[b]
H_2	423	1255	9.3
HCl	538	—	—
CH_4	550	1130	8.0
C_2H_4	601	—	—
i-C_3H_8	628	1046	7.5
C_2H_4	680	—	—
i-C_4H_{10}	683	967	6.9
H_2O	697	950	6.2
C_6H_6	759	—	9.3
CH_3OH	761	498	6.0
$C_2H_5NO_2$	773	—	—
CH_3SH	784	—	—
CH_3CN	787	—	—
$C_6H_5CH_3$	794	975	9.2
CH_3COOH	796	—	
CH_3COCH_3	823	—	—
NH_3	854	816	4.8
$(CH_3)_2NH$	923	—	—

[a] Data from Reference 75.
[b] Data from Reference 69.

would be observed in the PICI spectra. However, if the reagent gas were ammonia [ΔH(Reaction 7.12) = 854 − 794 = +60 kJ/mol], then the reaction would be endothermic and would not be observed. If the difference in PA is large (strongly exothermic), then there is a substantial excess energy in the $[M+H]^+$ cation and fragmentation may also occur. Thus, the degree of fragmentation increases as the PA of the reagent gas decreases (e.g., methane).

It should be understood that the sample molecule can have ∼4–8 kJ/mol of excess energy not accounted for in the preceding calculation due to the thermal energy that the sample molecule may acquire from the ion source wall or in the course of its chromatography. Therefore slightly endothermic reactions may be observed. It is noted that an entropy change for equilibrium processes such as Reaction 7.12 are usually small and can be neglected for this discussion (70).

There are no specific reagent gases that involve hydride abstraction as the major or sole ionization reaction. Many PICI spectra of organic compounds show both species. Compounds with significant hydrocarbon nature, such as fatty acids and long-chain methyl esters, show abundant peaks for both $[M+H]^+$ and $[M-H]^+$ cations. The thermochemistry of hydride abstraction reactions (Reaction 7.13) can be calculated in a similar manner as outlined above using hydride ion affinities (HIAs) instead of PAs [ΔH (Reaction 7.13) = HIA (reagent cation) − HIA (sample cation)]; that is, if the hydride ion affinity of the reagent cation ($[C]^+$) is higher than the hydride ion affinity of the cation formed by loss of H^- of the sample molecule ($[M-H]^+$), then the reaction is exothermic and would occur (75). As an example, if the reagent gas was methane and the sample molecule was toluene [ΔH (Reaction 13) = −1130 − (−975) = −155 kJ/mol], the reaction is exothermic and would occur.

The charge exchange Reaction (7.14) produces $[M]^{+\bullet}$ cations characteristic of EI. Consequently the fragmentation observed for charge exchange reactions are similar to those observed in EI. However, the $[M]^{+\bullet}$ cations produced by EI have a distribution of internal energies of 0–70 eV, whereas the $[M]^{+\bullet}$ cations have discrete internal energies defined by the exothermicity of the charge exchange Reaction (7.14). The exothermicity of Reaction 7.14 is determined by the ionization energy (IE; Table 7.4) of the sample molecule [M] less the recombination energy (RE; Table 7.5) of the reagent cation [ΔH (Reaction 7.14) = IE (sample molecule) − RE (reagent cation)]. The recombination energy is simply the energy released when an electron recombines with a cation to form a neutral species. The ionization energy is the energy required to remove an electron. Reagent gases for charge exchange include the noble gases, nitrogen, carbon dioxide, carbon monoxide, and hydrogen. As an example, if the reagent gas were hydrogen and the sample molecule were toluene [ΔH (reaction) = 9.2 − 9.3 = −0.1 eV], the reaction would be exothermic and would occur. In contrast, if the reagent gas were methane and the sample molecule were toluene [ΔH (reaction) = 9.2 − 8.0 = +1.2 eV], the reaction would be endothermic and would not occur (69). Helium has a reaction energy (RE) equal to 24.6 eV, and most organic molecules have IEs in the range 7–12 eV. Correspondingly, when He^+ is used as the reagent cation, complete fragmentation often results.

When reactions such as proton transfer, hydride abstraction, and charge exchange are not thermodynamically favorable, cluster reactions (e.g., Reaction 7.15) are sometimes observed. For example, in ammonia PICI an intense $[M+H+NH_3]^+$ cation is observed at $M + 18$ μ and sometimes $[M+H+(NH_3)_n]+$ cations are observed where $n = 1, 2, \ldots$; in methane PICI the presence of $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ cations are observed at M+29 and M+41, respectively; in isobutane PICI the presence of $[M+C_3H_3]^+$ and $[M+C_4H_9]^+$ cations are observed at M+39 and M+57, respectively. When the sample molecule pressure is sufficiently high, dimers ($[2M+H]^+$) are also produced (Reaction 7.18). Kebarle has shown that dimer cations are particular prevalent in the PICI spectra of sample molecules such as amines and alcohols since they are capable of forming hydrogen bonds (78). If the cluster cations that are formed by Reactions 7.15 and 7.18 are unstable, subsequent fragmentation may also be observed (Reaction 7.17).

7.4.3 Instrumentation

The GC/PICIMS technique was developed primarily by Arsenault (79) and Munson (80). The combination of the PICI technique with gas chromatography was a natural outgrowth from the GC/EIMS technique. The general considerations outlined for GC/EIMS (Section 7.3) are the same for GC/PICIMS. However, there are three major differences between the GC/EIMS and GC/PICIMS instrumentation that deserve attention:

1. Since PICI utilizes the principle of cation/molecule reactions (Reactions 7.10–7.15) between sample molecules (10^{-3}–10^{-4} Torr) and a high pressure (0.2–2 Torr) plasma of reagent gas, a specially designed CI source is required. The CI source is usually an EI source that can operate at high pressure. In order to operate at these high pressures the CI source must be "tight"; thus, the apertures of the electron filament entrance and the ion exit must be kept small (Figure 7.14). Typical examples for the electron filament entrance aperture are EI (3 mm^2) and CI (0.3 mm^2) and for the ion exit aperture are EI (1.5 mm^2) and CI (0.15 mm^2). The ion exit aperture can have a major effect on CI sensitivity and may need to be increased or decreased. Most modern CI sources can be altered to accommodate aperture changes.
2. Since the CI source is at a high pressure, such as 0.5 Torr, electrons with 70 eV energy penetrate a short distance into the source. The high pressure of the CI source requires a much higher electron energy (200–500 eV) to have an over all efficiency—expressed in amperes of ion current per microgram of sample—approaching that of an EI source.
3. MS instruments that operate in the CI mode must be differentially pumped. When the gas chromatographic effluent and the reagent gas (1–5 mL/min) enters the ion source and then exits through apertures in the CI source into the vacuum envelope surrounding it, the CI source housing pressure should

be at $\sim 10^{-4}-10^{-5}$ Torr. The pressure in the MS analyzer region must be maintained at $\sim 10^{-5}-10^{-6}$ Torr. Diffusion pumps or turbomolecular pumps with pumping speeds in the range of 1000–1500 L/s are used to maintain these pressures. Diffusion and turbomolecular pumps are backed by rotary pumps. It is important that the MS analyzer remain at low pressure since the cations must traverse a fairly long path in a highly focused beam. At higher pressures the beam scatters and deteriorates MS performance.

Certain source parameters, such as the repeller voltage, reagent gas pressure and ion source temperature, can markedly affect the PICI sensitivity and the appearance of the PICI spectra. Each of these parameters effect the residence time of the cations in the ion source, the kinetics and the thermochemistry of the cations and thus, effects the yields of cations present in Reactions 7.10–7.15. The repeller is used to move cations that are formed in the ion source out of the ion source. A positive voltage (0–10 V) is applied to a repeller plate, which is located inside the CI source (Figure 7.14) and is usually tuned to produce the maximum number of reagent gas ionizing species (~7–10 V). The repeller has a major effect on sensitivity. If the repeller is set too low, some low-abundance cations may be absent in the PICI spectrum. The effect of ion source pressure on the PICI spectrum of di-*n*-butyl phthalate is shown in Table 7.6 to illustrate this parameter. At low methane pressure no chemical ionization occurs and the PICI spectra resemble EI spectra (80). There is typically a range of pressures where the change in the overall appearance of the PICI mass spectra is insignificant. For this example, this is observed in the range 0.3–0.5 Torr. At 1.0 Torr there is an increase in abundance of the high-mass cations and a decrease in abundance of the low mass cations. This is due to greater collisional stabilization of the $[M+H]^+$ cation, from increased residence time in the ion source and thus, less fragmentation of the $[M+H]^+$ cation. The pressure dependence of

TABLE 7.6 Effect of Ion Source Pressure on Methane PICI Spectrum for Di-*n*-Butyl Phthalate[a]

Source Pressure (Torr) \ *m/z*	Percent of Base Peak						
	149	177	205	223	$[M+H]^+$ 279	$[M+C_2H_5]^+$ 307	$[M+C_3H_5]^+$ 319
0.1	100	—	7	4	—	—	—
0.3	100	13	97	9	32	3	2
0.5	100	17	99	10	39	4	3
1.0	49	7	100	6	81	14	9

[a]GC/PICIMS spectrum obtained under the following conditions: GC conditions—column DB-1 (30 m × 0.320 mm); film thickness 5.00 μm; carrier gas helium at 25 cm/s; oven program 45°C for 3 min, then 10°C/min to 300°C for 12 min.; injector port 265°C; sample 1 μL at 2000 μg/μL; solvent methylene chloride; samples were injected in the splitless mode (0.75 min load). MS conditions—mass range 50–500 μ; electron energy 200 eV; ion source temperature 200°C; repeller 7.0 V; GCMS interface temperature 250°C.

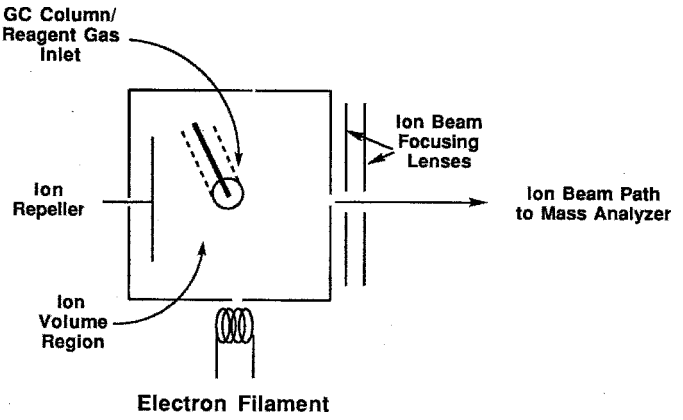

FIGURE 7.14 Diagram of chemical ionization source.

PICI spectra is seldom investigated—however, by increasing the reagent gas pressure, from 0.3 to 1.0 Torr, a factor of 2 is gained in the abundance of the $[M+H]^+$ cation at m/z 279. At pressure higher than 1.0 Torr, there is a decrease in the total abundance of cations formed due to deterioration of the MS performance. The effect of ion source temperature on the PICI spectrum of di-*n*-butyl phthalate is shown in Table 7.7. An increase in the ion source temperature reduces the abundance of the higher-mass cations $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ and increases the abundance of the lower-mass cations. If the ion source temperature could be increased to higher temperatures, the PICI spectrum of di-*n*-butylphthalate would probably mimic the EI spectrum due to excessive cation fragmentation caused by thermal effects (81). When the ion source temperature was changed from 150° to 300°C, the abundance of cations at m/z 149 and 205 reversed. The cation–molecule reaction for these cations is summarized in Figure 7.15.

Since m/z 149 is produced from m/z 205, as the temperature is increased one would expect to observe an increase in the abundance of m/z 149 and a decrease in the abundance of m/z 205. The cations' residence time in the ion source are all decreased at higher temperatures. In addition to the effect of

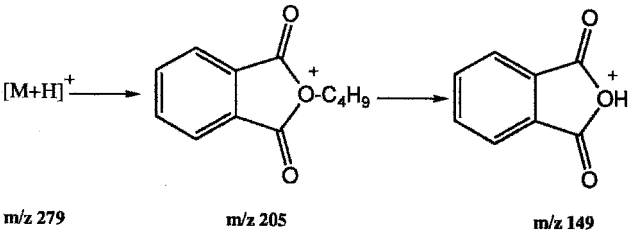

FIGURE 7.15 Reaction scheme of di-n-butyl phthalate.

TABLE 7.7 Effect of Ion Source Temperature on Methane PICI Spectrum for Di-*n*-Butyl Phthalate[a]

Source Temperature (°C) \ *m/z*	Percent of Base Peak						
	149	177	205	223	$[M+H]^+$ 279	$[M+C_2H_5]^+$ 307	$[M+C_3H_5]^+$ 319
150	78	12	100	9	51	7	4
200	100	17	99	10	39	4	3
250	100	15	85	8	33	1	—
300	100	16	55	5	11	—	—

[a]GC/PICIMS spectrum obtained under the following conditions: GC conditions—column DB-1 (30 m × 0.320 mm); film thickness 5.00 μm; carrier gas helium at 25 cm/s; oven program 45°C for 3 min, then 10°C/min to 300°C for 12 min.; injector port 265°C; sample 1 μL at 2000 μg/μL; solvent methylene chloride; samples were injected in the splitless mode (0.75 min load). MS conditions—mass range 50–500 μ; electron energy 200 eV; reagent gas methane 1.2×10^{-4} Torr (ion source housing); ion source pressure 0.5 Torr; repeller 7.0 V; GCMS interface temperature 250°C.

temperature on cation abundance, chemical decomposition of thermally labile molecules may also be a problem. There is always a compromise between a lower temperature, which would allow the ion source to contaminate quickly, and a higher range, that would promote higher energy collisions or decomposition. Only by investigating temperature and pressure effects can any potential problems be clarified.

7.4.4 Chromatographic Carrier Gas Substituted as the Reagent Gas

The choice of chromatographic carrier gas and CI reagent gas in GC/PICIMS are very important topics. The correct chromatographic carrier gas should be used to maximize column efficiency while the correct reagent gas should be used to maximize PICI sensitivity. The chromatographic carrier gas can be substituted as the reagent gas in some cases. Methane, helium, and hydrogen are three examples of chromatographic carrier gases that can be used also as the reagent gas with little effect on chromatographic resolution. In Figure 7.16 are shown three chromatographic traces of a five-component mixture. Figure 7.16a shows the results using methane as the chromatographic carrier gas and the reagent gas. Figure 7.16b shows the same mixture but with helium as the chromatographic carrier gas and methane as the reagent gas. The mass spectra were identical for these two combinations—that is, the cations at $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ were present usually in the ratio of 100–20–5, respectively. With helium as the chromatographic carrier gas and the PICI reagent gas (Figure 7.16c), charge exchange PICI spectra were obtained which resembled EI data. Note that the relative retention times and the resolution were the same for all three combinations. When hydrogen is used (not shown) as both carrier gas and PICI reagent gas, the PICI spectra contain a mixture of proton transfer $[M+H]^+$ and proton abstraction $[M-H]^+$ peaks.

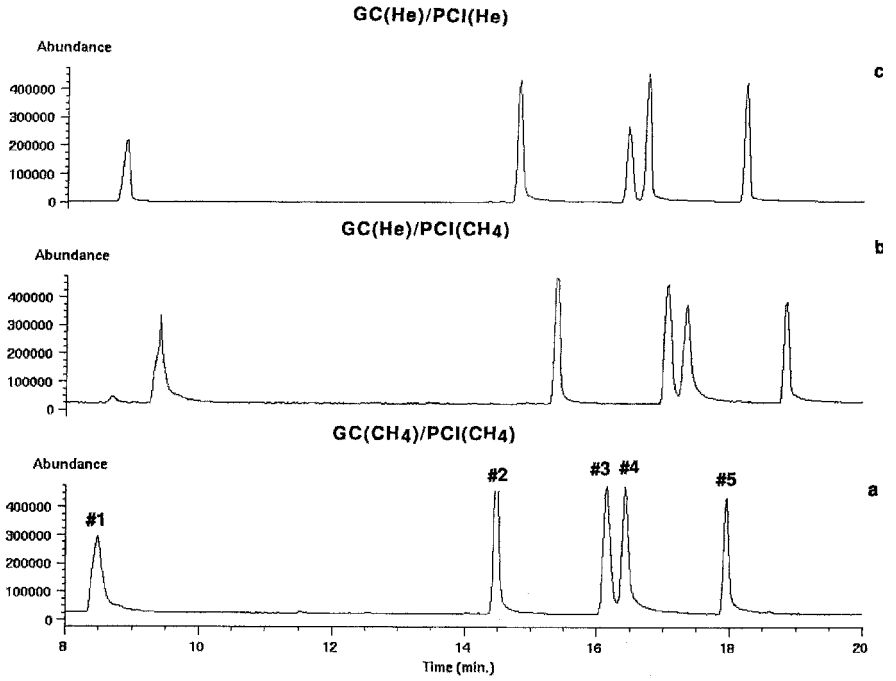

FIGURE 7.16 (a) The TIC chromatograms of a five-component mixture of (1) (*N*-nitrosodimethylamine), (2) [bis(2-chloroethyl)ether], (3) [bis(2-chloroisopropyl)ether], (4) (*N*-nitrosodi-*n*-propylamine), and (5) [bis(2-chloroethoxy)methane]. GC/PICIMS spectrum obtained under the following conditions: GC conditions—column DB-1 (30 m × 0.320 mm); film thickness 5.00 μm; carrier gas methane at 25 cm/s; oven program 45°C for 3 min, then 10°C/min to 300°C for 12 min; injection port 265°C; sample 1 μL at 2000 μg/μL; solvent methylene chloride; samples were injected in the splitless mode (0.75 min load). MS conditions—mass range 50–500 μ; electron energy 200 eV; reagent gas methane 1.2×10^{-4} Torr (ion source housing); ion source pressure 0.5 Torr; repeller 7.0 V; GCMS interface temperature 250°C; ion source temperature 200°C. (b) Same as (a) except the carrier gas was helium at 25 cm/s. (c) Same as (a) except the carrier gas was helium at 25 cm/s and the reagent gas was helium at 0.5 Torr. (Masucci and Caldwell, unpublished data.)

7.4.5 Helium Chromatographic Carrier Gas and Different Reagent Gases

It is more typical in GC/PICIMS to use helium as the chromatographic carrier gas while varying the reagent gas. In Figure 7.17 are shown the total-ion current (TIC) traces of a five-component mixture using three different reagent gases. The reagent gas for Figure 7.17a was ammonia, for Figure 7.17b it was isobutane, and for Figure 7.17c it was methane. The mixture consisted of two nitroso compounds (components 1 and 4) and three chloroether compounds (components 2, 3, and 5). The chromatographic carrier gas was helium for all three examples. Note

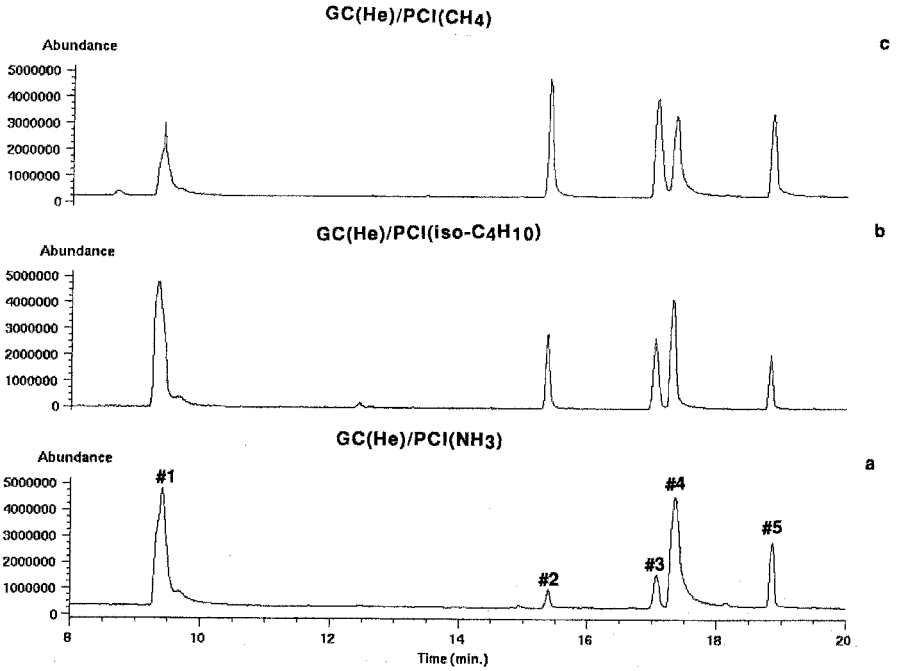

FIGURE 7.17 (a) TIC chromatograms of a five-component mixture of (1) (*N*-nitrosodimethylamine), (2) [bis(2-chloroethyl)ether], (3) [bis(2-chloroisopropyl)ether], (4) (*N*-nitrosodi-*n*-propylamine), and (5) [bis(2-chloroethoxy)methane]. GC/PICIMS spectrum obtained under the following conditions: GC conditions—column DB-1 (30 m $\times$ 0.320 mm); film thickness 5.00 μm; carrier gas helium at 25 cm/s; oven program 45°C for 3 min, then 10°C/min to 300°C for 12 min; injector port 265°C; sample 1 μL at 2000 μg/μL; solvent methylene chloride; samples were injected in the splitless mode (0.75 min load). MS conditions—mass range 50–500 μ; electron energy 200 eV; reagent gas ammonia 1.2×10^{-4} Torr (ion source housing); ion source pressure 0.5 Torr; repeller 7.0 V; GCMS interface temperature 250°C; ion source temperature 200°C. (b) Same as (a) except the reagent gas was isobutane at 0.5 Torr. (c) Same as (a) except the reagent gas was methane at 0.5 Torr. (Masucci and Caldwell, unpublished data.)

that the absolute retention times and the chromatographic resolution were the same for all three combinations. Also note that components 1 and 4 are much more pronounced than components 2, 3, and 5 when ammonia is used as the reagent gas. This is an example of ammonia selectivity for the components in the mixture containing nitrogen functionalities. The nitroso compounds have proton affinities more similar to ammonia than the chloroether compounds (75). The transfer of a proton, from the NH_4^+ cation, to the chloroether compounds is not as exothermic as the proton transfer to the nitroso compounds. Thus, the abundance of the $[M+H]^+$ cations for the chloro-ether compounds is less with a decrease in sensitivity.

It is of interest to examine the mass spectra contained in the TIC peaks (Figure 7.17). Variation of reagent gases can be used for selective ionization, fragmentation, and detection of specific functional groups. For example, the PICI spectra of *N*-nitroso-di-*n*-propylamine (component 4) and bis(2-chloroethoxy) methane (component 5), using ammonia, isobutane, and methane as reagent gases, are shown in Figures 7.17 and 7.18, respectively. The reagent NH_4^+ cation transfers a proton to *N*-nitroso-di-*n*-propylamine to produce a $[M+H]^+$ cation at *m*/*z* 131 and a cluster $[M+NH_4]^+$ cation at *m*/*z* 148 (Figure 7.17a). Note also the presence of the dimer at $[2M+H]^+$. The $t\text{-}C_4H_9^+$ cation from isobutane produces a $[M+H]^+$ cation at *m*/*z* 131, while the CH_5^+ and the $C_2H_5^+$ cations from methane produce a $[M+H]^+$ cation at *m*/*z* 131. The $C_2H_5^+$ and $C_3H_5^+$ cations cluster to produce peaks at *m*/*z* 159 and 171, respectively. We can compare these spectra with those shown in Figure 7.19. Bis(2-chloroethoxy)methane produces only a $[M+NH_4]^+$ cation at *m*/*z* 190 (Figure 7.19a) with no significant fragments. When isobutane is used as the reagent gas a small $[M+H]^+$ cation at *m*/*z* 174 and fragments are observed. When methane is used only fragments are observed in the PICI spectrum.

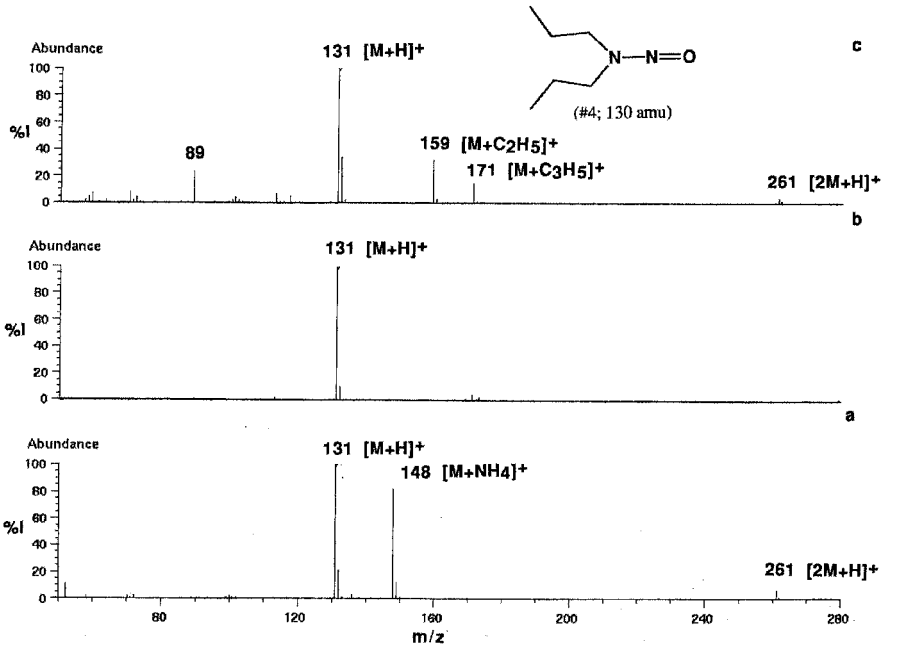

FIGURE 7.18 (a) PICI spectrum of *N*-nitroso-di-*n*-propylamine (peak 4 in Figure 7.17a) with ammonia as the reagent gas. (b) PICI spectrum of *N*-nitroso-di-*n*-propylamine (peak 4 in Figure 7.17b) with isobutane as the reagent gas. (c) PICI spectrum of *N*-nitroso-di-*n*-propylamine (peak 4 in Figure 7.17c) with methane as the reagent gas. (Masucci and Caldwell, unpublished data.)

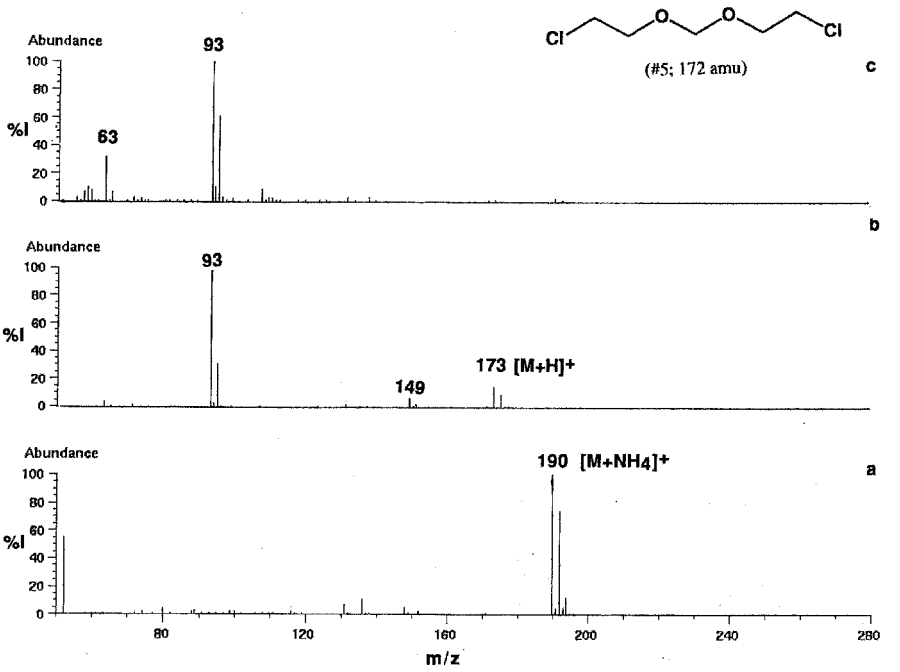

FIGURE 7.19 (a) PICI spectrum of bis(2-chloroethoxy)methane (peak 5 in Figure 7.17a) with ammonia as the reagent gas. (b) PICI spectrum of bis(2-chloroethoxy) methane (peak 5 in Figure 7.17b) with isobutane as the reagent gas. (c) PICI spectrum of bis(2-chloroethoxy) methane (peak 5 in Figure 7.17c) with methane as the reagent gas. (Masucci and Caldwell, unpublished data.)

7.4.6 Hydrocarbon Positive-Ion Chemical Ionization Reagent Systems

Hydrocarbon reagent gases such as methane and isobutane are the most common reagent gases that produce characteristic and abundant cations for determining molecular weights and fragments for structural elucidation. For methane, the following cation/molecule reactions describe the sequence of events in the ion source (64):

$$CH_4 + e^- \rightarrow CH_4^{+\bullet}, CH_3^{+\bullet}, CH_2^{+\bullet}, CH^{+\bullet}, \cdots + 2e^- \tag{7.21}$$

Methane is ionized by an electron bombardment to produce a series of radical cations. The major radical cations are $CH_4^{+\bullet}$ and $CH_3^{+\bullet}$, which represent approximately 90–95% of the total ionization at ~1 Torr. The $CH_2^{+\bullet}$ radical cation is produced at a much lower concentration. These radical cations react with methane to produce the following cations:

$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet \tag{7.22}$$

$$CH_3^{+\bullet} + CH_4 \rightarrow C_2H_5^+ + H_2 \tag{7.23}$$

$$CH_2^{+\bullet} + CH_4 \rightarrow C_2H_3^+ + H_2 + H^\bullet \quad (7.24)$$

$$C_2H_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad (7.25)$$

Thus, the PICI spectrum of methane contains cations at m/z 17 ($[CH_5]^+$), m/z 29 ($[C_2H_5]^+$), and m/z 41 ($[C_3H_5]^+$) with small relative concentrations of $C_2H_3^+$, $C_3H_7^+$, $C_2H_2^+$, $C_3H_3^+$, $C_3H_4^+$, and $C_4H_9^+$. When this series of cations at $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$ is observed in the methane PICI spectra the molecular weight can be stated with confidence. The CH_5^+ cation can protonate all organic compounds exothermically. When proton transfer occurs with considerable exothermicity, the $[M+H]^+$ cation contains appreciable energy and fragmentation occurs (see Figure 7.18c). Other hydrocarbons such as methane-d_4 (66), propane (66), *n*-hexane (82), and *n*-octane (82), have also been used as reagent gases. A review on unusual PCI reagents has been published with an emphasis on the analytical applications (83).

An interesting artifact that appears in methane PICI spectra involves the following set of reactions (84):

$$[M + H]^+ + [M] \rightarrow [2\ M + H]^+ \quad (7.26)$$

$$[2\ M + H]^+ \rightarrow [F + M]^+ + [N] \quad (7.27)$$

where F is a fragment cation and N is neutral. As an example, the methane PICI spectrum of 1,1′-methylenebis[pyrrolidine] contains cations at m/z 84 ($[F]^+$; 100%), at m/z 153 ($[M-H]^+$; 3%), at m/z 155 ($[M+H]^+$; 2%), and at m/z 238 ($[F+M]^+$; 3%). Note that the $[F+M]^+$ peak is a relatively abundant peak when compared to the $[M-H]^+$ and $[M+H]^+$ cations. The $[F+M]^+$ peak could be mistaken as an impurity or the protonated molecular cation.

7.4.7 Amine Positive Ion Chemical Ionization Reagent Systems

Amine reagent gases such as ammonia and methylamine are the most common gases that produce characteristic and abundant cations for determining molecular weights. The following cation–molecule reactions describe the sequence of events, for ammonia, in the ion source (85):

$$NH_3 + e^- \rightarrow NH_3^{+\bullet}, NH_2^{+\bullet}, \cdots + 2e^- \quad (7.28)$$

Ammonia is ionized by electrons to produce a series of radical cations. The major radical cation is $NH_3^{+\bullet}$, which represents approximately 90% of the total ionization. The $NH_2^{+\bullet}$ radical cation is produced at a much lower concentration. These radical cations react with ammonia to produce the following cations:

$$NH_3^{+\bullet} + NH_3 \rightarrow NH_4^+ + NH_2^\bullet \quad (7.29)$$

$$NH_2^{+\bullet} + NH_3 \rightarrow NH_4^+ + NH^\bullet \quad (7.30)$$

$$NH_4^+ + NH_3 \rightarrow N_2H_7^+ \quad (7.31)$$

$$N_2H_7^+ + NH_3 \rightarrow N_3H_{10}^+ \quad (7.32)$$

Thus, the PICI spectrum of ammonia contains primarily a cation at m/z 18 ($[NH_4]^+$) with small relative concentrations of cations at m/z 35 ($N_2H_7^+$) and at m/z 52 ($N_3H_{10}^+$). When this series of cations at $[M+H]^+$ and $[M+NH_4]^+$ are observed in the ammonia PICI spectra, one can confidently state the molecular weight (see Figure 7.18a). The ammonia PICI technique is selective for phosphorous and nitrogen bases in the presence of many common solvents. The NH_4^+ cation does not protonate water, acetone, ethylacetate, methanol, ethanol, halogenated hydrocarbons, tetrahydrofuran, and ethylether (75). A more general review of ammonia PICI has been published (85).

7.4.8 Applications–Structure Elucidations and Quantification

Structure elucidation of an unknown sample molecule is probably best accomplished utilizing hydrocarbon-type reagent gases. Hydrocarbons such as methane and isobutane give structural and molecular weight information. Methane PICI produces a series of cations at m/z M+1, M+29, and M+41 and fragments. Therefore, if a series of cations is observed in the methane PICI spectrum that have m/z differences of 28 and 40, then the molecular weight of the unknown can be defined with strong confidence. If uncertainty remains concerning the molecular weight, nitrogen-containing reagent gases can be used. Ammonia PICI spectra will typically contain M+1 and/or M+18 cations and fragments; thus a mass difference of 17 μ will establish the molecular weight. Combination charge exchange/chemical ionization (Ar/H_2O) reagent gases are useful (86). This type of combination provides soft ionization by H_3O^+ to produce $[M+1]^+$ cations and hard ionization by Ar^+ to produce fragments. The methodology of structure elucidation by PICI has been published elsewhere (69). The major fragmentation pathway for the $[M+H]^+$ cations is elimination of a stable neutral molecule [X] (Reaction 7.17). Table 7.8 presents some common neutral loss fragments observed in PICI. Some sample molecules show multiple neutral losses.

In some cases combined GC/isotopic exchange PICI can be used in structural studies. Under favorable GC/PICIMS conditions hydrogens bonded to heteroatoms like thiols, amines, amides, carboxylic acids, phenols, and alcohols undergo rapid isotopic exchange for deuterium in the CI source. Reagent gases such as D_2O (87,88), ND_3 (89,90), and CD_3OD (91,92) have been utilized. Since this approach is commonly used to differentiate isomers, there are many examples in the literature. For example, the molecular structure of four unknown halogenated compounds have been determined using NH_3/ND_3 PICI (93). The shift in the $[M+H]^+$ cations indicated that these unknowns were probably secondary amines. Three of the structures were unequivocally determined since synthesized standards showed identical retention times and isotopic exchange PICI patterns.

Quantitative measurement of a known analyte present in a complex matrix is a common application of GC/PICIMS (94–96). A deuterium-labeled analog or a homologous sample molecule is usually added as an internal standard to account for sample losses during workup and gas chromatographic separation. In order to achieve maximum sensitivity, a reagent gas is chosen such that one or more

TABLE 7.8 Common Neutral Loss Fragments Observed in Reaction 7.17

$[M+H-X]^+$	[X]
$[M+1-128]^+$	HI
$[M+1-80]^+$	HBr
$[M+1-36]^+$	HCl
$[M+1-20]^+$	HF
$[M+1-18]^+$	H_2O
$[M+1-32]^+$	CH_3OH
$[M+1-46]^+$	CH_3CH_2OH
$[M+1-90]^+$	$(CH_3)_3SiOH$
$[M+1-34]^+$	H_2S
$[M+1-48]^+$	CH_3SH
$[M+1-27]^+$	HCN
$[M+1-58]^+$	$(CH_3)_2CO$
$[M+1-17]^+$	NH_3
$[M+1-31]^+$	CH_3NH_2

cations that are characteristic of the sample molecule and the internal standard are produced in high yield. These cations may be any of those in Reactions 7.10–7.18 and/or fragments. Quantitative measurement by GC/PICIMS is usually done in the selective-ion or multiple-ion monitoring mode or over a limited mass range to achieve the highest sensitivity.

A typical example of a pharmacokinetic investigation is the quantitative determination of γ-hydroxybutyric acid (GHB) in plasma and urine samples (97). Plasma and urine GHB samples were converted to γ-butyrolactone (GBL), and GBL was subsequently concentrated using a headspace solid-phase microextraction technique. GC/PICI methane was used for the quantitative analysis. A deuterium-labeled D_6-GBL compound was used as the internal standard. The mass spectrometer was operated using selected ion monitoring for two $[M+H]^+$ cations (m/z 87 and 93) characteristic for both GBL and D_6-GBL. The calibration curve was linear over a plasma GHB range of 1–100 μg/mL with correlation coefficients of 0.999. The calibration curve was linear over a urine GHB range of 5–150 μg/mL with correlation coefficients of 0.998. For between-day and for within-day precision, the coefficient of variations were similar (<5%). The method described by these authors (97) produced the required precision, accuracy and sensitivity to assay GHB for purposes of therapeutic drug monitoring. There are many other examples in the literature of quantitative GC/PICIMS (94–102).

7.5 GAS CHROMATOGRAPHY/NEGATIVE-ION CHEMICAL IONIZATION MASS SPECTROMETRY

7.5.1 Advantages of Negative Ion Chemical Ionization

The chemical ionization process produces both positive (cations) and negative (anions) ions and thus, an alternative to positive-ion chemical ionization mass

spectrometry (PICI) is negative-ion chemical ionization mass spectrometry (NICI). A general review of NICI has been published by Harrison (69,103) and others (104–106). There are two major types of NICI techniques used today: electron capture and acidity/hydrogen bonding techniques. While molecular weight information can be obtained from both NICI techniques, there is typically little structural information from either. The NICI electron-capture technique can be 10–100 times more sensitive than the NICI acidity/hydrogen-bonding technique or the PICI technique. Therefore, the NICI electron-capture technique has been utilized extensively for quantification studies (104–106). Gas chromatography/negative-ion chemical ionization mass spectrometry (GC/NICIMS) studies are typically performed with helium as the chromatographic carrier gas and a variety of reagent gases. Changing the detection mode has no effect on the chromatographic resolution—thus, all conclusions drawn from the GC/PICIMS technique (Section 7.4) will apply here.

The NICI electron capture technique requires that a gaseous mixture consisting of a reagent gas [R_1] (e.g., methane, isobutane, or ammonia) for the production of thermal electrons and the sample molecule [M] of interest be present in the ion source. When the anion mode of detection for the mass spectrometer is set up, only anions are observed. In a typical experiment, the reagent gas [R_1] is ionized by electrons at ~200 eV. Cations, anions, and radicals are produced along with thermal electrons (Reaction 7.33). As the name implies thermal electrons are low-energy electrons, which have a very narrow distribution of energies (ranging from 0 to ~5 eV). These thermal electrons react with sample molecules [M] to produce radical (attachment) anions $[M]^{-\bullet}$ (Reaction 7.34), dissociative attachment anions $[M-X]^-$ (Reaction 7.35) and ion pair anions $[X]^-$ (Reaction 7.36). The sample molecules are not ionized to any extent by the high-energy electrons since these electrons are converted to low energy quickly. The abundance of $[M]^{-\bullet}$, $[M-X]^-$ and $[X]^-$ anions depends on the reagent gases and the sample molecules. Only certain types of sample molecules (e.g., nitroaromatic compounds, polyhalogenated compounds, and highly conjugated π systems bearing electron-attracting substituents) will react via Reactions 7.34–7.36:

$$e^-(200\ \text{eV}) + [R_1] \rightarrow C_p^+ + C_p^- + 2e^-(\text{thermal}) \tag{7.33}$$

$$e^-(\text{thermal}) + [M] \rightarrow [M]^{-\bullet} \tag{7.34}$$

$$e^-(\text{thermal}) + [M] \rightarrow [M-X]^- + [X] \tag{7.35}$$

$$e^-(\text{thermal}) + [M] \rightarrow [M-X]^+ + [X]^- + e^- \tag{7.36}$$

The NICI acidity/hydrogen-bonding technique requires that a gaseous mixture consisting of a reagent gas [R_1] (e.g., methane, isobutane, or ammonia) for the production of thermal electrons, a reagent gas [R_2] for the generation of reactive anions and the sample molecule [M] of interest are present in the ion source. The second reagent gas [R_2] is used such that the thermal electron reacts with [R_2] to produce reactive anions $[C]^-$ (Reaction 7.37). This reactive anion $[C]^-$ can react with the sample molecules [M] to produce proton abstraction

anions $[M-H]^-$ (Reaction 7.38) and/or cluster adduct anions $[M+C]^-$ (Reaction 7.39). The abundance of these anions is controlled by anion/molecule reactions and ultimately depends on the reagent gases and the sample molecules. Sample molecules that contain an acidic proton such as alcohols, carboxylic acids and phenols work well:

$$e^-(\text{thermal}) + [R_2] \rightarrow [C]^- \tag{7.37}$$

$$[C]^- + [M] \rightarrow [M-H]^- + [C+H] \tag{7.38}$$

$$[C]^- + [M] \rightarrow [M+C]^- \tag{7.39}$$

If the analyte pressure is sufficiently high, dimers are also produced (Reactions 7.40 and 7.41).

$$[M+C]^- + [M] \rightarrow [2\,M+C]^- \tag{7.40}$$

$$[M-H]^- + [M] \rightarrow [2\,M-H]^- \tag{7.41}$$

More details on how $[M]^{-\bullet}$ and $[M-H]^-$ anions are generated in the NICI technique will be discussed in Sections 7.5.2–7.5.5. The anions that are produced via both NICI techniques usually produce very little or no fragmentation.

Figure 7.20 illustrates an example of the methane/methyl iodide NICI spectrum of 5,5-dimethyl-1,3-cyclohexanedione. In this example, methane is the $[R_1]$ reagent gas that produces thermal electrons upon electron ionization. Methyl iodide is the $[R_2]$ reagent gas used to generate the reactive iodide anion ($[I]^-$) at m/z 127. This $[I]^-$ anion reacts with 5,5-dimethyl-1,3-cyclohexanedione ($[M] = 140\ \mu$) to produce the cluster $[M+I]^-$ peak at m/z 267 (Reaction 7.14). Note there is no fragmentation for this NICI technique.

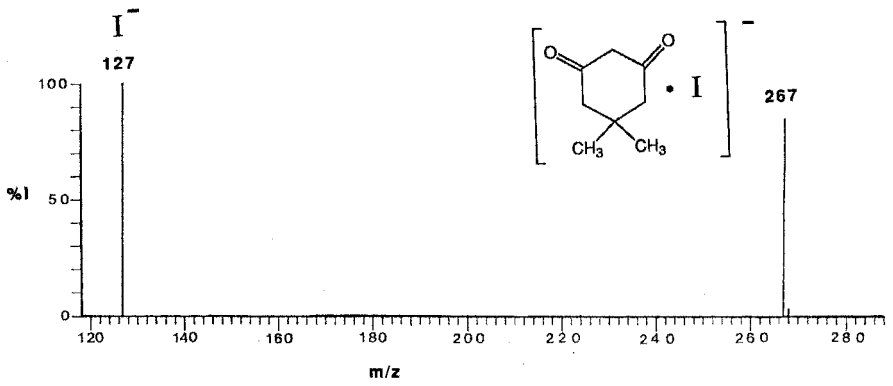

FIGURE 7.20 (a) (CH_4/CH_3I) NICI spectrum of 5,5-dimethyl-1,3-cyclohexanedione. The following conditions were used: reagent gas methane at 0.4 Torr; reagent gas methyl iodide at 10^{-6} Torr; electron energy 500 eV; repeller at −9 V; mass range 50–500 μ; ion source temperature at 115°C. A solids probe was used as the inlet. (Masucci and Caldwell, unpublished data.)

7.5.2 Kinetic and Thermodynamic Considerations

Knowledge of kinetics and thermochemistry can predict and rationalize many properties of chemical ionization and reduce the effort of trial-and-error searching for optimum analytical conditions. The electron attachment anions $[M]^{-\bullet}$ (Reaction 7.34), the dissociative electron attachment anions $[M-X]^-$ (Reaction 7.35), and the ion pair anions $[X]^-$ (Reaction 7.36) are all produced as a result of electron/molecule reactions. These reactions can have rate constants in the range 10^{-8}–10^{-7} cm^3 molecule^{-1} s^{-1} (69,103). Since the electron has a higher mobility than a cation (or an anion), these rate constants are considerably higher than those in PICI. As a result of these rate constants, the NICI electron attachment technique can have sensitivity increases of 10–100 over those of the NICI acidity/hydrogen-bonding technique or the PICI technique. The proton abstraction anions $[M-H]^-$ (Reaction 7.38) proceed with rate constants on the order of $1–4 \times 10^{-9}$ cm^3 molecule^{-1} s^{-1} (69,103). Note that these rate constants are the same as the PICI rate constants. Thus, there is no gain in sensitivity from changing ionization modes.

At ion source pressures on the order of 0.5 Torr and ion source temperatures of approximately 373 K, the rate constants for electron attachment and proton abstraction suggest that there are an adequate number of collisions in the ion source to permit equilibria to be sufficiently established. This is a prerequisite in order to assume a Boltzmann distribution of internal energies of the anions (or cations). Thus thermochemical data, such as electron affinities and the proton affinities of anions, can be used to calculate the energetics of these reactions (75). The cluster adduct anion $[M+C]^-$ (Reactions 7.39–7.41) have third-order rate constants. Where comparisons can be made, the magnitude of positive- and negative-mode third-order rate constants are similar (69,103). The clustering reactions are important in NICI spectra for polar compounds in the presence of polar molecules such as water and alcohol.

The electron affinity (EA) of an anion is defined as the lowest energy required to remove an electron. An equivalent definition is the affinity of the sample molecule [M] for an electron:

$$[M] + e^- \rightarrow [M]^{-\bullet} \tag{7.42}$$

The EA of many compounds have been measured (69,75,103,105–111) and selected examples are presented in Table 7.9. If a sample molecule has a negative EA, such as benzene, then the electron attachment reaction (Reaction 7.42) is not energetically favorable. However, if a sample molecule has a positive EA, such as perfluorotoluene, then the electron attachment reaction is energetically favorable. From the EA data in the literature, structural features that must be contained in a molecule in order for that molecule to have a positive EA may be extrapolated. For example, compounds with functional groups containing highly conjugated π systems such as large polycyclic aromatic rings or polycarbonyl type species should have positive EAs. Aromatic rings bearing electron-attracting substituents such as nitro, carbonyl, cyano, and trifluoromethyl groups should have positive

TABLE 7.9 Examples of Electron Affinities (kJ/mol) and Anion Proton Affinities (kJ/mol)

Species (B)	EA(B)[a]	Species (BH)	PA(B^-)[b]
H^-	75	H_2	1675
$O^{-\bullet}$	142	·OH	1599
CH_3^-	754	CH_4	1744
Cl^-	349	HCl	1395
Br^-	327	HBr	1354
HO^-	176	H_2O	1635
$C_6H_6^{-\bullet}$	<0		
CH_3O^-	156	CH_3OH	1592
c-$C_5H_5^-$	161	*c*-C_5H_5	1481
CH_3S^-	183	CH_3SH	1493
MCF3-$C_6H_4NO_2^{-\bullet}$	136		
CH_2CN^-	141	CH_3CN	1560
$C_6H_5CH_2^-$	87	$C_6H_5CH_3$	1593
CH_3COO^-	296	CH_3COOH	1459
$C_6H_5NO_2^{-\bullet}$	97		
$CH_3COCH_2^-$	180	CH_3COCH_3	1544
NH_2^-	72	NH_3	1689
$C_6F_5CF_3^{-\bullet}$	164		

[a] Data from References 75 and 109.
[b] Data from Reference 75.

EAs. Two or more halogens on an aromatic ring or polyhalogenated hydrocarbons typically have positive EAs (106–111).

While a positive EA is a necessary criterion for production of the radical anion $[M]^{-\bullet}$, the observation of a $[M]^{-\bullet}$ anions in NICI spectra also depends on the lifetime of these radical anions in the NICI source. In other words, molecules with positive electron affinities may not be observed because they lose an electron (autodetachment) due to collisions with the reagent gas. Autodetachment of the radical anion is important for small molecule and organic molecules with small positive (<50 kJ/mol) EAs (75,110,111). Also, once a sample molecule has captured an electron, it may follow a dissociative electron attachment reaction pathway to produce $[M-X]^-$ anions (Reaction 7.35) or may produce ion pair $[X]^-$ anions (Reaction 7.36) (69,103).

Once the radical anion is formed by Reaction 7.42, it may not be observed due to a charge exchange Reaction (7.43):

$$[M]^{-\bullet} + [N] \rightarrow [N]^{-\bullet} + [M] \tag{7.43}$$

The charge exchange Reaction (7.43) will be exothermic provided the EA of [N] is greater than the EA of [M]. For example, if one was interested in detecting nitrobenzene ([M] = $C_6H_5NO_2$) in the presence of perfluorotoluene ([N] = $C_6F_5CF_3$), then ΔH (Reaction 7.43) = EA[M] − EA[N] = 97 − 164 =

−67 kJ/mol. The reaction is strongly exothermic and the $[M]^{-\bullet}$ anion would not be observed. Charge exchange reactions are important when oxygen-containing impurities are present in the reagent gas or perfluoro-containing calibration gas is present as background in the GCMS.

The anion proton affinity (gas phase acidity) is generalized by Reactions 7.44 and 7.45. The fundamental concept of anion proton affinity (acidity) is well defined in the gas phase (69,75,103,110,111).

$$[M] \rightarrow [M-H]^- + [H]^+ \tag{7.44}$$

$$[C]^- + [H]^+ \leftarrow [C+H] \tag{7.45}$$

The addition of Reactions 7.44 and 7.45 is simply Reaction 7.38, and thus the energetics for the abstraction of a proton from the sample molecule [M] by the reactive anion $[C]^-$ can be calculated by comparing the gas-phase acidities of the sample molecule to the gas-phase acidities of [C+H]; that is, ΔH (Reaction 7.38) = PA (sample) − PA (C+H). If the reactive anion has an anion proton affinity (gas-phase acidity) greater than that of the sample molecule, then the NICI reaction can take place. For example, if the reactive anion were Cl^- ([C+H] = HCl) and the sample molecule were toluene ([M] = $C_6H_5CH_3$), then ΔH (Reaction 7.38) = 1593 − 1395 = +198 kJ/mol. The reaction would be endothermic and would not occur. However, if the reactive anion were NH_2^- ([C+H] = NH_3) and the sample molecule were toluene [ΔH (Reaction 7.38) = 1593 − 1689 = −96 kJ/mol], the reaction would be exothermic and would occur. If the reaction is strongly exothermic, there is substantial excess energy in the $[M-H]^-$ anion and fragmentation could occur or the electron could detach.

7.5.3 Instrumentation

Instrumentation considerations for NICI are the same as those outlined for PICI (Section 7.4.3). A tight CI source, an electron energy that can operate at ∼200 eV and good pumping speeds are required. To operate in the NICI mode, the polarity of the voltages applied to the repeller, the lenses of the ion source and the detection system are reversed. A negative voltage is applied to the repeller and positive voltages are applied to the lenses. Under these conditions anions are expelled from the ion source. The ion exit aperture has a major effect on NICI sensitivity and may need to be increased or decreased. Most modern NICI sources can be altered to accommodate aperture changes. The detection system varies from instrument to instrument and has different configurations. The changeover from positive to negative mode is usually computer-controlled and selected through the data system software.

An interesting artifact observed in quadrupole mass spectrometers is the appearance of a $[M+CH_5]^-$ anion in the NICI spectra when methane is used as a reagent gas (112). In some quadrupole mass spectrometers set up for negative mode, cations can be formed outside the ion source and are recorded along with

anions that were formed in the ion source. These spectra contain peaks that are produced by cations and anions.

The repeller voltage, the sample molecule to reagent gas ratio, the ion source pressure, and temperature can influence the sensitivity of the NICI technique and the appearance of the NICI spectra. These parameters more strongly influence the NICI technique than the PICI technique. The repeller has the same effect on sensitivity as described for the PICI technique; if the repeller is set too low, the overall sensitivity significantly decreases and/or low abundant anions may be absent in the NICI spectrum. The NICI spectrum of α-chlordane was investigated with different concentrations of methylene chloride and varying amounts of α-chlordane (113). The results indicated that the $[M+Cl]^-/[M]^{-\bullet}$ ratio varied with the α-chlordane/methylene chloride ratio. The effect of ion source pressure on the sensitivity and NICI abundances for α-chlordane has been studied over the range 0.1–0.3 Torr. There is usually an increase in sensitivity as the ion source pressure is increased. This increase in sensitivity has been attributed to an increase in collisional stabilization of the anion. At higher reagent gas pressure (>1 Torr), the sensitivity of NICI generally decreases, which is attributable to deterioration of the mass spectrometer performance. In general, the ion source pressure has a large effect on sensitivity; however, the appearance of the NICI spectrum changes slightly with pressure. Most NICI experiments are conducted with ion source temperatures in the range 100–250°C. Table 7.10 lists shown the effects of ion source temperature on the NICI spectrum of *p*-toluic acid (114).

As the temperature is increased, the overall sensitivity of the NICI technique decreases. The ion counts for the radical anion decreased by a factor of ~2. By increasing ion source temperature, the abundances of low mass fragments increase relative to the $[M-H]^-$ anion at m/z 135. Thus, the ion source temperature has a significant effect on the appearance of NICI spectra and the sensitivity. The abundance of the cluster anion (e.g., $[2\ M-H]^-$) decreases with increasing temperature and increases with increasing ion source pressure.

7.5.4 Electron-Capture Techniques

Gas chromatography/electron-capture negative-ion chemical ionization mass spectrometry (GC/ECNICIMS) has become widely used for quantification of

TABLE 7.10 Effect of Ion Source Temperature on NICI Spectrum of *p*-Toluic Acid

Source Temperature (°C) \ *m/z*	Percent of Base Peak						
	$[M-H]^-$						$[2M-H]^-$
	91	118	135	150	156	171	271
190	3	5	100	7	8	12	50
220	16	5	100	5	3	1	3
250	36	6	100	10	2	—	—

Source: Reference 114.

compounds at low concentration levels in complex matrices (115–125). The EC technique shows very large variations in sensitivity among different compound types, thus permitting specific compound detection in complex matrices. As noted (Section 7.5.3), small changes in the concentrations, in the ion source pressure and temperature from one analysis to another can have significant effects on the sensitivity and fragmentation in ECNICI spectra.

GC/ECNICI analytical quantification procedures usually contain many of the following steps. An internal standard is added to the complex matrix to correct for losses during sample preparation, clean up steps and GCMS analyses. Complex biological matrices, such as urine or plasma, are extracted into organic solvents and purified by chromatographic methods. As a result of certain functional groups (e.g., ROH and RCO_2H), these isolated samples may have unfavorable gas chromatographic properties leading to tailing peaks and/or unfavorable mass spectrometric properties leading to insensitive detection. The sample is generally derivatized to improve gas chromatographic properties (peak symmetry, volatility, thermal stability by replacing any active hydrogen atom: –OH, $–NH_2$, –NHR, –SH) in a molecule with a trimethylsilyl group. Examples of derivatization to improve electron-capture properties include the use of pentafluorobenzoyl chloride for reaction with phenols and amines, tetrafluorophthalic anhydride for reaction with amines and pentafluorobenzaldehyde for reaction with aromatic amines (125). In order to achieve maximum sensitivity, a reagent gas is chosen such that only the molecular anions $[M]^{-\bullet}$ of the sample molecule and the internal standard are produced. Quantitative measurement by GC/ECNICMS is usually done in the selective-ion monitoring mode. The peak area ratios of the molecular anion of the sample versus that of the internal standard are compared with the calibration curve in which the peak area ratios of the standard versus that of the internal standard were plotted versus their concentrations.

The combination of limited fragments, high sensitivity, and selectivity makes GC/ECNICI ideal for quantification studies of metabolites or drug substances from physiological fluids. A typical example of a pharmacokinetic investigation is the quantitative determination of triamcinolone acetonide (TAA) in human bronchoalveolar lavage fluid (117). The isolated samples were converted to their corresponding C21 acetate derivatives and purified using adsorptive chromatography prior to GC/ECNICMS analysis. A heptadeuterated analog of TAA was used as the internal standard with methane as the reagent gas and helium as the carrier gas. Calibration curves were linear over a range of concentrations of TAA from 0 to 12.3 ng/mL. A detection level of approximately 6 pg/mL could be readily detected in 2-mL aliquots of the fluid with $<10\%$ error. The method was suitable for the determination of deposition pattern and in vivo kinetics of TAA in human airways following inhalation of the steroid.

7.5.5 Acidity and Hydrogen-Bonding Techniques

The acidity/hydrogen-bonding NICI technique gives primarily proton abstraction anions $[M-H]^-$ (Reaction 7.38) and/or cluster adduct anions $[M+C]^-$ (Reaction 7.39). Exactly what anion is observed in the NICI spectrum depends on the

reactive anion $[C]^-$ and the sample molecule. The NICI spectra can be rationalized or predicted utilizing thermochemical data (69,75,103,110,111,126). Many different reagent gases can be used to generate reactive anions $[C]^-$. A variety of gases or gas combinations are listed in Table 7.11 that when reacted with thermal electrons produce reactive anions $[C]^-$ (Reaction 7.37). A qualitative prediction of the concentration of these anions in the CI source is also given.

The analytical potential of the NICI acidity/hydrogen bonding technique can be best understood by considering an example where methane is used for the production of thermal electrons and one of the halogen gases listed in Table 7.11 is used for the generation of a reactive halide anion ($[C]^- = [X]^- =$ F, Cl, Br, I). The sample molecule in this example contains an acidic proton ($[M] = [MH]$). Common experimental conditions include an ion source temperature of $\sim$100°C, an ion source pressure of $\sim$1 Torr, and small superimposed repeller electric fields (7–9 V). Under these conditions, an approach toward equilibrium can be obtained. The following reactions can take place in the CI source (Figure 7.21). Each of the reaction pathways is denoted, and an asterisk further denotes a short-lived excited intermediate complex. The NICI spectra obtained from this experiment contain either only the $[M-H]^-$ anion or the $[M+C]^-$ anion or a mixture of both. Nucleophilic displacement (S_N2) reactions are not considered here.

The gas-phase thermochemistry of the halide anions X^- (F,Cl,Br,I) with a given MH sample molecule is highly dependent on the halide anion radius. For

TABLE 7.11 Reagent Gases and Their Dissociative Attachment Anions

Species	Reactive Anions $[C]^-$	Yield of Anions
NH_3	H^-, NH_2^-	Medium
H_2O	H^-, HO^-	Medium
N_2O	$O^{-\bullet}$, NO^-	Medium
O_2	$O^{-\bullet}$, $O_2^{-\bullet}$	Low
RONO	RO^-, NO_2^-	High
$RONO_2$	NO_2^-	High
H_2S	H^-, HS^-	Medium
RSSR	RS^-	Low
HCN	CN^-	Medium
NF_3	F^-	High
SO_2F_2	F^-	High
SF_6	F^-	High
CCl_4	Cl^-	High
CF_2Cl_2	Cl^-	High
CF_3Br	Br^-	High
CH_3I	l^-	High
CH_3CN/H_2O	CN^-, $^-$CH2CN	Medium
N_2O/H_2	HO^-	Medium

Source: References 69, 103, 104, and 127.

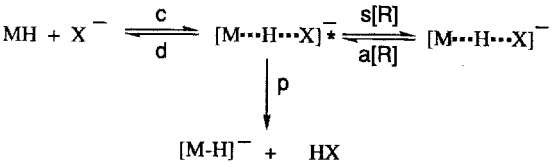

FIGURE 7.21 NICI reaction scheme where MH = sample molecule, R = methane, X = F, Cl, Br, I, and the reaction path notation is c = condensation, d = dissociation, s[R] = collisional activation, and p = proton transfer.

TABLE 7.12 Halide Binding Energies[a] (kJ/mol) and Anion Proton Affinities[b] (kJ/mol) of a Variety of Functional Groups

Species [MH]	F^{-c}	Cl^{-d}	Br^{-e}	I^{-e}	ΔH°_{acid}
CH_4	—	—			1744
NH_3	—	44		31	1689
C_6H_6	—	42		38	1677
H_2O	98	62	53	42	1635
$C_6H_5CH_3$					1593
CH_3OH	124	59		47	1592
CH_3CN	67	56	54	50	1560
HF	161	91	71	63	1554
$(CH_3)_2CO$	—	57	—	50	1544
$C_6H_5NH_2$	131	—	—	54	1533
H_2S	145	—		37	1469
C_6H_5OH	173	109			1461
CH_3CO_2H	185	90		71	1459
$(CN)_2CH_2$	—	—		79	1405
HCl	251	99	82	60	1395
$(CF_3)_3COH$	242			97	1388
HBr	272	123	87	67	1354
HI	301	128	106	71	1315

[a] Values obtained for reaction $[MH + X]^- \rightarrow X^- + HM$, namely, D(X−HM).
[b] Values obtained for reaction $[MH] \rightarrow [M-H]^- + H^+$, namely, ΔH°_{acid}.
[c] Data from Reference 132.
[d] Data from Reference 133.
[e] Data from Reference 126.

example, the gas-phase basicity of the halide anions decreases with increase of anion radius (128). Thus, proton transfer from MH to F^- (Figure 7.21; reaction path c and p) is exothermic for many organic acids ($\Delta H^\circ_{acid} > 1555$ kJ/mol) while proton transfer from MH to I^- is endothermic for many organic acids (Table 7.12). Since the extent of fragmentation depends primarily on the proton transfer exothermicity, it is reasonable to assume that fragmentation in halide NICI spectra should decrease with increase of anion radius for a given MH (see Figure 7.21). The binding energy of the hydrogen-bonded $[M\cdots H\cdots X]^-$ anion

decreases with an increase of ion radius (129–131). Generally, when comparing binding energies to a given X^- anion, binding energy increases with the gas-phase acidity of MH. This is shown in Table 7.12.

While there are exceptions to these generalizations (130,131), several conclusions can be drawn from these observations. When F^- is used as the reactive anion, the NICI spectra of organic acids ($\Delta H^\circ_{acid} > 1555$ kJ mol^{-1}) will contain large yields of the proton transfer $[M-H]^-$ anion and some fragmentation. When Cl^- or Br^- is used as the reactive anions, the NICI spectra will contain a mixture of the proton transfer $[M-H]^-$ anion, fewer fragment anions and the hydrogen-bonded $[M\cdots H\cdots X]^-$ anion. When I^- is used as the reactive anion, the NICI spectra will contain large yields of the hydrogen-bonded $[M\cdots H\cdots X]^-$ anion, no fragmentation or $[M-H]^-$ anion (see Figure 7.20).

7.6 DEVELOPING TRENDS IN GAS CHROMATOGRAPHY/MASS SPECTROMETRY

7.6.1 Multidimensional (Gas Chromatography)^m/(Mass Spectrometry)^n

Multidimensional techniques such as (gas chromatography)^m/(mass spectrometry)^n-GC^m/MS^n, where m or n equals 1, 2, and so on,—can improve the sensitivity, separation and selectivity of trace-level compounds in complex biological and environmental matrices. In analyses where the lowest detectable amount is limited by interference from endogenous components in the matrix, performance can be improved by using different ionization techniques or by increasing the separation at the chromatographic stage or by increasing the selectivity at the mass separation stage or by a hyphenated combination of all methods. The advantages of different ionization modes have been pointed out in previous sections (Sections 7.3–7.5). Interest in two-dimensional gas chromatography has grown (134–137). One important advantage of the GC × GC technique is the ability to inject large quantities of samples on the first column and then divert a fraction of the effluent to the second column. Thus, the chromatographic resolution is maintained. The analyte exiting the second column, which reaches the mass spectrometer, is much purer than with single gas chromatographic separations and ion source contamination is reduced. The specificity of the analysis in GC × GC is also improved greatly by establishing two retention times per component. This is achieved by using dissimilar stationary phase, for example, two columns with stationary phases selected for volatility and polarity retention mechanism is an effective method for separating complex mixtures. One important advantage of the MS × MS is its versatile platform for a broad range of experiments to provide sensitive and selective analysis of complex mixtures (138). Several multidimensional hyphenated experiments are possible where different ionization modes are used in combination with two dimensional chromatography and tandem mass spectrometry techniques (139–145). Several examples are described below.

Frysinger and co-workers (145) published an interesting application of comprehensive two-dimensional chromatography (GC × GC) coupled with a

single-stage quadrupole mass spectrometer for the analysis of petroleum samples. A thermal modulator was used to provide GC × GC capability (146). The major components of the thermal modulator tube are a modulator tube and a rotating slotted heater. Serial connection of the GC columns was accomplished using the modulator tube. To desorb the analyte and inject samples into the second column, the slotted heater was rotated at a known frequency over a thick-film stationary-phase section of the modulator tube. The process continued until all the analyte from the first column was injected onto the second column. Since the separation in the second dimension was fast relative to the first dimension, the scan speed of the MS must be fast. They required approximately 12 scans per second to obtain reasonable baseline separation; however, their quadrupole MS was limited to 2.43 scans per second. To overcome this limitation, they could either slow down the chromatography in the second dimension or operate the MS in a SIM mode. In a complex petroleum sample, even with these limitations outlined above, they were able to identify minor components, distinguish members of homologous series, and characterize order peak patterns of related components.

Since the GC peak widths are very narrow (∼0.2 s) in the second -dimension of comprehensive GC × GC applications, the use of TOF mass spectrometers have gained considerable interest in (139,142). The TOF mass filter is an integrating rather than a scanning detector; therefore the acquisition rate is limited only by the ion pulse frequency and the spectrum storage speed. The current computer technology allows a spectrum storage speed on the order of 500 spectra per second (45) with mass accuracy of approximately 5 ppm (46). Van Deursen and co-workers (142) have coupled a comprehensive GC × GC system with a TOF mass spectrometer for the analysis of petroleum samples. It was possible to detect sulfur- and oxygen-containing compounds in petroleum samples by selecting appropriate cation fragments. The TOFMS was operated over a mass range 40−280 μ at an acquisition rate of 50 spectra/s. The effects of modulator temperature and frequency, column temperature programming rates, and carrier-gas velocity on the performance of comprehensive two-dimensional chromatography (GC × GC) coupled to a TOF mass spectrometer have been studied (139).

An interesting alternative to comprehensive (GC × GC)/TOFMS has been investigated using a parallel GC approach (147). In this experiment, the injected sample was split between two comparable GC columns. The effluent was recombined, from the two columns, prior to TOFMS analysis. In this manner, the chromatographic axis had twice the number of peaks, and thus the chemical selectivity of the analysis had increased without increasing the analysis time. The parallel method may be suitable for improving the high-speed GCMS analysis of complex mixtures by not requiring extensive calibration models containing every possible chemical in the complex sample.

7.6.2 High-Speed Gas Chromatography/Mass Spectrometry

The long analysis times normally associated with GCMS separations can be a major limitation for applications in which higher throughput or rapid real-time monitoring is required, or where thermal degradation of analytes occurs over the

course of the separation. Since chromatographic efficiencies can be quite high in GC, it is possible to speed up the analysis and still allow sufficient separation of mixture components. In the past, the major limitation to speeding up the separation was related more to scanning speed restrictions with conventional magnetic sector and quadrupole mass spectrometers, than to chromatographic considerations. Recent improvements in quadrupole scan speeds now allow acquisition rates of up to 20 scans per second over a selected mass range that is more than sufficient for detection of peaks only 0.5–2 s wide. Sector instruments can be configured with fast duty cycle, focal plane detectors, as previously described, to allow simultaneous recording of all scanned masses. Additionally the reintroduction over the last few years of time-of-flight MS (TOFMS) analyzers with orthogonal acceleration and array detectors have further extended the possibilities for high-speed GCMS (HSGC/MS) as acquisition rates of transients can approach several thousand per second, yielding tens to hundreds of spectra per second (148).

Several groups have reported on the use of HSGC/MS (149–152). These generally fall into two categories and have resulted in two commercial instruments representative of each. In one case, short narrow capillary columns (30–100 cm length, $<$0.1 mm i.d.) are utilized to decrease runtimes while maintaining reasonable efficiencies. These experiments are typically run at 1–3 mL/min of carrier-gas flow with runtimes of 0.5–5 min. Column inlet pressures of several bars are typically used to maximize linear velocity while decreasing runtimes. The separation of an eight-component hydrocarbon mixture in less than one second has been reported using this approach (153). A commercial instrument is manufactured by LECO Corp (St. Joseph, MI) based generally on this first approach using a TOFMS analyzer for rapid scanning.

The second method utilizes larger diameter megabore capillary columns (0.53 mm i.d.) with very high flowrates of up to 30 mL/min through the use of a supersonic molecular beam (SMB) interface for HSGC/MS (152). With this approach, a slightly longer column is used to maintain efficiency (3–5 m) while the SMB allows for removal of the majority of the carrier gas while transmitting the analyte stream to the spectrometer operating much like a jet separator, described previously. The SMB also has the effect of cooling the vibrational energy of the molecules resulting in what is referred to as “cold EI” spectra in which the molecular ion abundance is frequently enhanced over conventional EI sources. This allows for better verification of molecular mass and can also improve library searching in some cases. HD Technologies (Manchester, UK) manufactures an instrument that incorporates the SMB interface with TOFMS analyzer.

In an effort to quantitate the increase in GCMS analysis speed, one author has suggested the descriptors “fast,” “very fast,” and “ultrafast” (154). These are based on the definition of the new term, *speed enhancement factor* (SEF), which is defined as the product of column length reduction and the increase in carrier-gas linear velocity for the chromatographic separation. Using this description, SEFs of 5–30, 30–400, and 400–4000 correspond to fast, very fast, and ultrafast,

respectively. These factors are normally achieved by decreasing column length or column internal diameter and/or increasing inlet gas pressures while maintaining sufficient chromatographic resolution.

Other variations of HSGC/MS include the use of membrane introduction mass spectrometry (MIMS) for the detection of trihalomethanes (THMs) in drinking water (155). The THMs in water diffuse through a length of hollow fiber membrane, where they were cryotrapped using liquid nitrogen, then rapidly heated and directed onto a GC column for separation and detection using a quadrupole MS operating at about 8 scans/s. The separation of 4 THMs was performed in less than 1.6 min. This approach allows analyses of about 20 water samples per hour, which is much faster than the conventional purge-and-trap method.

There has also been a report of a miniature field-portable instrument capable of performing HSGC/MS analysis using a special ion trap/time-of-flight analyzer (156). The instrument has potential uses for point of release environmental monitoring as well as detection of chemical agent exposure for military applications. It is quite likely that the uses of these devices will expand as the technology improves and the need for definitive, sensitive, monitoring methods grow.

7.6.3 Novel Ionization Methods for Gas Chromatography/ Mass Spectrometry

GCMS has traditionally utilized either electron or chemical ionization because of their direct compatibility with vaporized analytes and their efficiency at producing ions for a large variety of organic molecules. However, it is frequently advantageous, especially with complicated sample matrices to achieve selective ionization of analytes. This has the effect of maximizing analyte response while minimizing signal from background or endogenous materials which often coelute in complex mixtures. Additionally, because of their enclosed volume designs, EI and CI sources can contribute to observed peak-tailing due to longer residence time of ions in the source region and slower response characteristics. This is even more critical when using HSGC/MS, in which analysis times are very short and any lingering of vaporized molecules in the source region will adversely affect the results.

One approach which has been reported is the use of surface ionization (SI) techniques with both conventional GCMS (157) and in combination with SMB HSGC/MS (152). In the SI method, the GC effluent stream is directed onto a heated metal surface, usually rhenium or tungsten maintained at a temperature of about 400–800°C. The surface is continuously exposed to a small amount of oxygen gas, which causes an oxide coating to be formed on the metal surface. Molecules in the analyte stream with low ionization potentials, such as amines, undergo an electron transfer to the metal oxide surface generating positive ions that are mass-measured using conventional quadrupole or TOF analyzers. In its simplest implementation, a direct probe, to which a short length of metal foil is attached, is introduced into the EI source region of a conventional GCMS instrument. A small amount of oxygen is bled into the ion source. Current is

passed through the metal foil to resistively heat it to the appropriate temperature. The chromatographic effluent is directed onto this heated surface causing formation of ions through several possible mechanisms, including electron transfer, generating a positively charged molecular ion; thermally induced dissociative ionization, which generates positive and negative fragment ions; proton transfer, which generates protonated molecular ions; and thermally induced chemical ionization, where reactive species are thermally generated that combine with analyte molecules to form charged products. By varying metal surface temperature, electric field, and gas pressure and composition, selective ionization of analytes is achieved. Easily ionized compounds include amines, quaternary ammonium salts, aminoalcohols, and hydrazines, while aliphatic compounds have little SI sensitivity. Also since SI is performed with an open source design it has a rapid response time and does not contribute to peak tailing. A surface ionization probe is now commercially available (Shimadzu Corp., Japan).

One demonstrated application of this method involved the detection of a series of drugs from urine (158). In this work a SMB GCMS instrument was used with SI to achieve low-ppb detection of drugs directly from urine. This was possible because of the selective nature of SI in which the nitrogenous drugs were preferentially ionized over other endogenous components in urine. Detection of cocaine at 1 ppb in spiked urine was reported using this method using selected ion monitoring and less that 1 μL of raw urine.

Another selective ionization approach involves the use of multiphoton ionization of analytes as they elute from the chromatographic column (159,160). Using a tunable laser, analytes can be specifically ionized by choosing the appropriate wavelength. This allows background components to be excluded in some cases if they have different UV absorption frequencies.

The detection and identification of both atomic and molecular species has traditionally been difficult by GCMS since ionization modes that generated both were not available and obtaining both types of information required running samples in different experiments using different equipment and procedures. Inductively coupled plasma MS has traditionally been used for determination of atomic species, but is too energetic to allow formation of molecular ions. The use of a gas-sampling glow discharge (GSGD) ionization source for GCMS has been reported that generates both atomic and molecular ions (161). In operation, a helium gas glow discharge is established in the ion source region of a TOFMS. The chromatographic effluent is introduced into the discharge, which is operated alternately in the atomic and molecular modes with a 50% duty cycle at a typical frequency of 10 Hz, which was sufficient for sampling the chromatographic peaks. The results show that signals could be obtained on a single GC run for both atomic composition and molecular mass spectra of eluting analytes. The analysis of a series of chlorinated hydrocarbons showed good similarity to conventional EI spectra while also allowing determination of the atomic composition using $^{35}Cl^{+}/^{12}C^{+}$ ratios for additional characterization. These analytes could easily be identified using this approach. If this method could be extended to larger organic

molecules, then further evidence of compound identity would be provided by atomic composition ratios.

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CHAPTER EIGHT

Qualitative and Quantitative Analysis by Gas Chromatography

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PART 1 QUALITATIVE ANALYSIS

8.1 DISCUSSION OF CHROMATOGRAPHIC DATA

Inherently, two important pieces of data can be obtained from a gas chromatograph. The output of the detector is either processed electronically or placed on a simple strip chart recorder. The first piece of data obtained is simply the time it took for a given component to travel through the column. This is the time from the point of injection to the maximum of the peak as it passes through the detector. This time is referred to as the absolute retention time t_R. It is this retention time information that is not used in qualitative analysis. The second important piece of information that is obtained is simply the size of the peak. Size data is discussed in Part 2 of this chapter, "Quantitative Analysis." A third piece of information that can be obtained from the chromatograph is the shape

of the peak. This is available only if the chromatogram is displayed for the individual. This information is lost in many of the electronic integrators that are in use today. The shape of the peak may give some information for both qualitative and quantitative analysis. This peak shape is discussed where it is important in both of these parts. The chromatogram also provides information about the chromatographic operation of the system and any degradation that may have occurred with time or with a particular sample.

8.2 IDENTIFICATION FROM GAS CHROMATOGRAPHIC DATA ONLY

8.2.1 Retention Data

Qualitative analysis by gas chromatography (GC) in the classical sense involves the comparison of adjusted retention data t'_R of an unknown sample with that of a known sample. The gas chromatographic technique has two negative aspects regarding qualitative analysis. The technique alone cannot confirm the presence of a single analyte molecule. Under a given set of conditions, any compound analyzed by GC has a characteristic retention time; however, this retention time is not unique—other compounds could have the same retention time. Likewise, nonvolatile molecules are not amenable to separation by GC (a molecule should have a vapor pressure of at least a 0.1 Torr for analysis by GC).

The alternative approach involves a combination and comparison of gas chromatographic data with data from other instrumental and chemical methods of

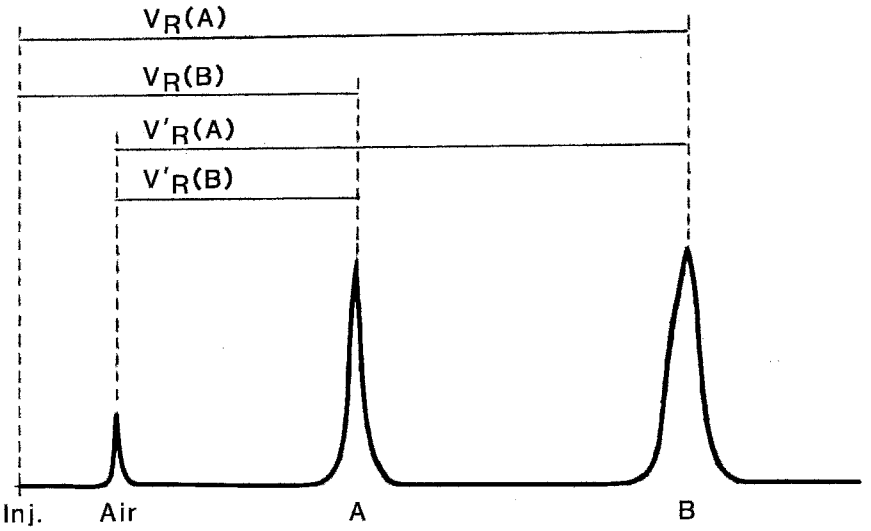

FIGURE 8.1 Chromatogram illustrating retention nomenclature: V_R = retention volume, V'_R = adjusted retention volume, $V_{A/B}$ = relative retention = $V'_{R(A)}/V'_{R(B)}$.

analysis. The simplest qualitative tool is simply the comparison of adjusted retention data from known and unknown samples. A chromatogram illustration of the commonly used retention nomenclature is given in Figure 8.1. The retention time t_R is the time elapsed from injection of the sample component to the recording of the peak maximum. The retention volume V_R is the product of the retention time and the flowrate F_c of the carrier gas. Generally, the adjusted retention time t'_R or adjusted retention volume V'_R and the relative retention $r_{a/b}$ are used in qualitative analysis. Adjusted retention time (volume) is the difference between retention time (volume) of the sample and an inert component (usually air) or some nonretained component (e.g., methane). The relative retention is the ratio of the adjusted retention time (or volume) of a standard to the adjusted retention time (or volume) of the unknown (see Chapter 1).

There are three fundamentals concerning retention times obtained on a given instrument with a given column operating under fixed operating conditions. These fundamentals must be known, understood, and believed before useful qualitative data can be obtained from gas chromatographic information only. The first and most important principle is simply that if the adjusted retention time of component A is equal to the adjusted retention time of an unknown component, this does not prove that the unknown component is component A. This is the major pitfall of qualitative analysis and is the statement that prevents gas chromatography from being an exceptional qualitative tool. The rest of the first part of this chapter is devoted to ways and means of supplementing retention data to obtain qualitative information about a sample. The second fundamental is simply that if the adjusted retention time of component A does not equal the adjusted retention time of an unknown component, then indeed with absolute certainty we can say that the unknown component is not component A. The third important fundamental is that if we have no discernible peak at the adjusted retention time of component A, we can say with certainly that no component A is present in the sample to our limits of detection.

Many factors must be considered in comparison of any retention measurements. The precision of the data generally depends on the ability of the instrument to control the temperature of the column and the flowrate of the carrier gas. A change in the temperature of approximately 30°C changes the retention time by a factor of 2. Thus, to maintain a 1% repeatability in retention measurements, one must hold the column temperature to within $\pm$ 0.3°C. A 1% change in the carrier-gas flowrate affects the retention time by approximately 1%.

Sample size also plays an important role (see Figure 8.2). If too much sample is introduced onto the column for its diameter and stationary-phase loading, "leading peaks" will appear. These leading peaks are distorted, giving a slow rise to the peak and a fast drop. As shown in Figure 8.2, the actual time of the peak maximum shifts to longer times, causing the retention time to actually increase for more of a particular component. This phenomenon is caused by column overload. This can be most apparent in gas–solid chromatography, where the action is simply a surface action. In gas–liquid chromatography it is more important at very low loadings of stationary phase on the column packing. Higher

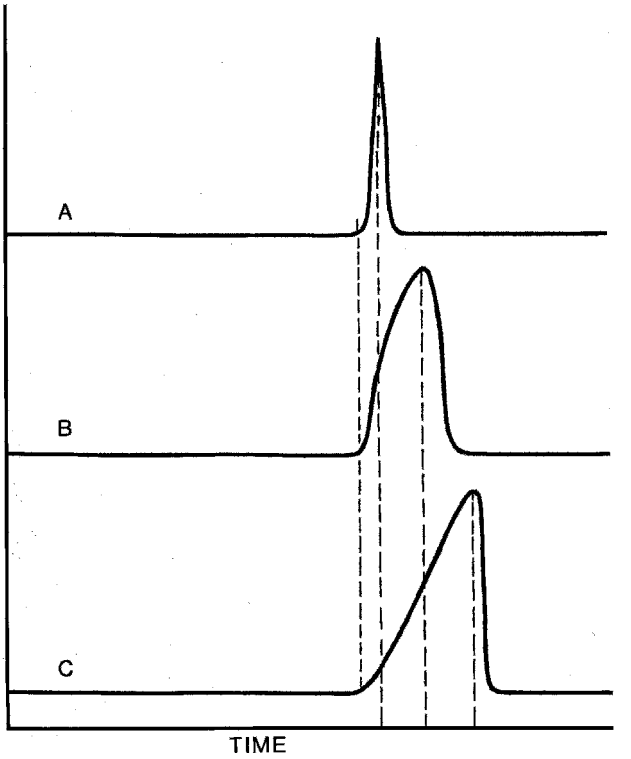

FIGURE 8.2 Effect of sample size on retention time: (a) column not overloaded; (b) column slightly overloaded; (c) column severely overloaded.

loadings will not cause column overload to occur as rapidly as the component amount in the sample is increased. In some cases the column temperature is operating above the boiling point of the component. Instead of seeing leading peaks, we actually see tailing peaks where the front edge of the peak is very sharp and the back edge of the peak slopes. In this case the retention time moves to shorter times under a column overload condition. In comparing retention times for qualitative analysis, one should be alert to this overload condition and test it simply by cutting the sample size in half and injecting the sample again. If retention times stay constant, both conditions could be said to be under a nonoverload situation. If the retention time changes for the reduced sample size, however, the sample size must be reduced once again to ensure that the system is operating in a nonoverload or ideal condition.

Attempts to compare retention times on two different columns of the same type can be difficult at best. Differences in packing density, liquid loading, activity of the support, age and previous use of the packing, and variations in the comparison of the column wall can lead to large differences in retention time measurement between the two columns. Thus tabulations of absolute retention times are not

of much value in qualitative analysis. However, there are a number of solutions to this dilemma. The first and simplest solution is the use of relative retention times. The relative retention of a component is simply its adjusted retention time divided by the adjusted retention time of a reference material. This is indicated in Figure 8.1, where the reference material is assumed to be peak B. Relative retention data are much less subject to variation from column to column and for slight changes in temperature and flow changes. It is also quite simple to obtain relative retention data.

8.2.2 Plot of Log Retention Time versus Carbon Number

A linear dependence exists between the logarithm of the retention volumes for compounds in homologous series and the number of carbon atoms in the molecule. This relationship has been shown to hold for many classes of compounds such as alkanes, olefins, aldehydes, ketones, alcohols, acetates, acetals, esters, sulfoxides, nitro derivatives, aliphatic amines, pyridine homologs, aromatic hydrocarbons, dialkyl ethers, thiols, alkyl nitrates, substituted tetrahydrofurans, and furan. A typical series of plots of the logarithm of the retention volume versus the carbon number is given in Figure 8.3. It must be reemphasized that this method of identification is valid only for members of a homologous or pseudohomologous series. However, if plots such as that shown in Figure 8.3 are known for a given column under a given set of operating conditions, this method can be extremely useful in helping to identify unknown components. In many cases the first member, and even in some cases the second member, of the series may deviate slightly from this strictly linear relationship. In general, however, one does not have a column so well defined at a fixed set of operating conditions that a large number of these curves are available. It is reasonably easy to obtain these curves because, strictly speaking, only two compounds in the series are needed to

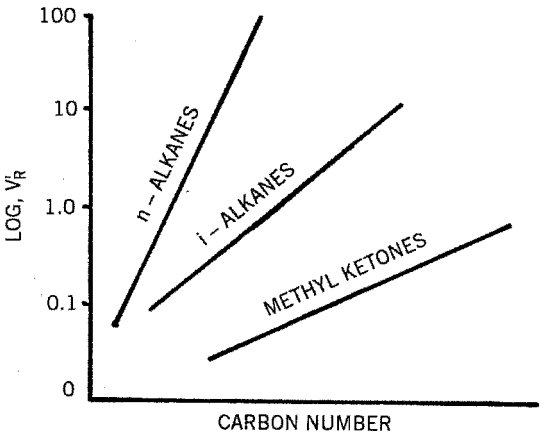

FIGURE 8.3 Logarithm of adjusted retention time versus carbon number.

define the curve. These curves can be quite useful in at least eliminating certain classes of compounds relative to known peaks in a chromatogram. For instance, if the retention time of an unknown peak falls between the seven- and eight-carbon straight-chain alkanes, it is impossible for the unknown to be a straight-chain alkane since fractional carbon atoms are not allowed in the molecule. This technique can eliminate a number of potential materials.

8.2.3 Kovats Index

Wehrli and Kovats (1) introduced the concept of the retention index to help confirm the structure of the organic molecules. This method utilizes a series of normal alkanes as a reference base instead of one compound as in the relative retention method. Identification can be assisted with the use of the retention index I:

$$I = 100N + 100\left[\frac{\log V'_{\mathrm{R}}(A) - \log V'_{\mathrm{R}}(N)}{\log V'_{\mathrm{R}}(n) - \log V'_{\mathrm{R}}(N)}\right] \tag{8.1}$$

where N and n are the smaller and larger n-paraffins respectively, that bracket substance A, and V'_{R} is the adjusted retention volume. The retention indices for n-alkanes are defined as 100 times the number of carbon atoms in the molecule for every temperature and for every liquid phase (e.g., octane = 800, decane = 1000).

In practice, the retention index is simply derived from a plot of the logarithm of the adjusted retention time versus carbon number times 100 (Figure 8.4). To obtain a retention index, the compound of interest and at least three hydrocarbon standards are injected onto the column. At least one of the hydrocarbons must elute before the compound of interest and at least one must elute after it. A plot of the logarithm of the adjusted retention time versus the Kovats index is constructed from the hydrocarbon data. The logarithm of the adjusted retention time of the unknown is calculated, and the Kovats index is determined from the curve (Figure 8.4).

Many factors can influence the Kovats index, which make it unreliable at times for characterization of gas chromatographic behavior, although it generally varies less than relative retention with temperature, flow, and column variation. For many, however, the Kovats index is the preferred method of reporting retention data.

A number of attempts have been made to correlate retention index and molecular structure (2). Success here can greatly enhance the use of the retention index in qualitative analysis.

8.2.4 Multiple Columns

The use of two or more columns improves the probability that the identity of an unknown compound is the same as that of a compound with identical retention times. However, these data alone are not conclusive proof. The reliability of the identification depends on the efficiency and polarities of the column used. With

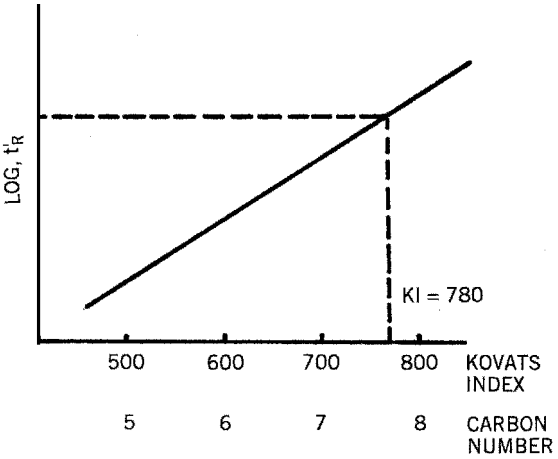

FIGURE 8.4 Plot of logarithm of adjusted retention time versus Kovats index.

TABLE 8.1 Pesticide Relative Retention Times

	Columns					
Pesticide	1	2	3	4	5	6
Lindane	0.44	0.46	0.47	0.44	0.74	0.81
Heptachlor	0.78	0.79	0.79	0.78	0.85	0.87
Aldrin	1.00	1.00	1.00	1.00	1.00	1.00
Dieldrin	1.88	1.84	1.83	1.93	2.70	3.00
Endrin	2.12	2.06	2.05	2.18	3.19	3.56
P,P'-DDT	3.19	3.10	3.03	3.50	3.63	4.07

Column Packing	Column Size	Temperature	Reference
1 3.8% UCW-09	7 ft × 2.2 mm i.d.	195°C	3
2 3% SE-30	6 ft × 2.2 mm i.d.	180°C	3
3 10% DC-200	6 ft × 4.0 mm i.d.	200°C	4
4 3% OV-1	5.9 ft × 4.0 mm i.d.	180°C	5
5 5% OV-210	6 ft × 2.2 mm i.d.	180°C	3
6 5% OV-210	6 ft × 4.0 mm i.d.	180°C	5

efficient columns the probability of having two or more components under one peak diminishes and the peaks are generally well resolved. Care must be taken in selecting columns to be certain that columns have different selectivities and not just different names. The McReynolds constants (see Chapter 3) must be compared and should be quite different for each column. Table 8.1 shows the relative retention times for a number of chlorinated pesticides on six different columns. From the relative retention data shown, it would certainly appear that the first four columns are handling the pesticides in basically the same fashion. If

two of these columns were selected to help confirm the identity of an unknown by using two different columns, therefore, we would expect these not to show differences and thus give a confirmation. In fact, four of these columns could be used and we could be quite convinced that the unknown is the same as the component whose retention time it matches on these four different columns. However, an examination of McReynolds constants (see Chapter 3) for these four columns certainly indicates that regardless of the name attached, which are trade names, the materials are essentially all the same. Indeed, they are methylsilicone polymers. Table 8.1 certainly indicates that the column OV-210 is a different column from the other four columns. However, this same piece of information could be determined very readily by published McReynolds numbers.

One problem in using retention time to identity unknown components occurs in a multicomponent mixture where more than one component in the mixture may have the same retention time on even two or three different columns. Laub and Purnell (6,7) have described a systematic technique of using multicomponent solvents in the gas chromatographic column to optimize separation of mixtures. This technique should not be overlooked in qualitative analysis since it can be fairly useful in spotting two or more components contributing to the same peak (see Chapter 4).

It should also be noted that in addition to retention time measurements obtained on two or more column systems, if reasonable care has been exercised, quantitative measures of the suspect compound should also correspond, thus providing additional secondary identification. In other words, regardless of what the unknown compound is, it cannot be a mixture of two components on one column and a single component on the second column without quantitative measure detecting this fact. The value of this particular observation is commonly ignored. Information on the structure of an unknown peak can be obtained from the difference in the retention indices on polar and nonpolar stationary phases:

$$\Delta I = I_{\text{polar}} - I_{\text{nonpolar}} \tag{8.2}$$

For a particular homologous series ΔI is a specific value that is determined by the character of the functional group(s) of the molecule. Takacs and coworkers (8,9) calculated the Kovats index for paraffins, olefins, cyclic hydrocarbons, and homologs of benzene on the basis of molecular structures. The index was divided into three additive portions: atomic index, bond index, and sample stationary-phase index components (see also Reference 2).

8.2.5 Relative Detector Response

8.2.5.1 Selective Detectors

Comparison of the relative detector response from two or more detectors can aid in the identification or classification of an unknown component. Generally the component is chromatographed on one column and the effluent split and fed to two or more detectors. Commonly used pairs of detectors are the phosphorus

and electron capture, flame ionization and radioactivity, and flame ionization and phosphorous detectors. The electron-capture detector allows the identification of substances containing atoms of phosphorous, oxygen, nitrogen, and halogens in a complex mixture while remaining quite insensitive to other substances. Flame photometric detectors are useful with phosphorus- or sulfur-containing compounds. The flame ionization detector (FID) is especially sensitive to virtually all organic materials, but especially hydrocarbons. (For a complete discussion of specific and nonspecific detectors, see Chapter 6.)

8.2.5.2 Molecular Weight Chromatography

The molecular weight of a component can be obtained through mass chromatography. This relies on two gas density detectors, two columns, and two carrier gases. A diagram of a typical mass chromatographic system is given in Figure 8.5. The sample is introduced into the injection chamber by syringe, gas-sampling valve, pyrolysis unit, or reaction chamber and trapped on two separate trapping columns. After the sample has been trapped, it is displaced from the traps by backflushing and heating and swept onto two matched chromatographic columns using two different carrier gases. The carrier gases are chosen on the basis of significant difference in molecular weight; for example, CO_2 (44 g/mol) and SF_6 (146 g/mol). The sample is then separated on the column and the eluate is passed through each gas density detector. Thus two peaks are recorded for each component (Figure 8.6). The molecular weight of a component is obtained from the ratio of the two peak heights or areas by use of the following equation:

$$\mathrm{MW} = \frac{K(A_1/A_2)(\mathrm{MW_{CG2}} - \mathrm{MW_{CG1}})}{K(A_1/A_2) - 1} \tag{8.3}$$

where K is an instrumental constant; A_1 and A_2 are the area responses of the unknown component from detectors 1 and 2, respectively; and $\mathrm{MW_{CG1}}$ and $\mathrm{MW_{CG2}}$ are the molecular weights of carrier gas 1 and carrier gas 2, respectively. In practice, A_1 and A_2 are measured for known compounds and K is determined for the experimental conditions. Then the molecular weight of the unknown is determined by obtaining its area ratio and using the K previously

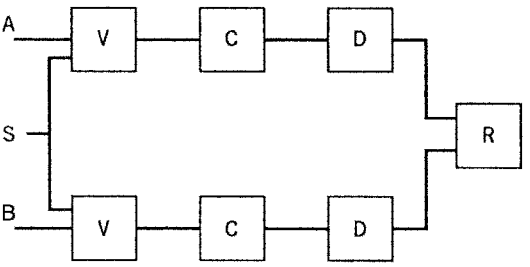

FIGURE 8.5 Mass chromatograph: A, carrier A inlet; S, sample inlet; B, carrier B inlet; V, valve–trap system; C, chromatographic column; D, gas density detector; R, recorder.

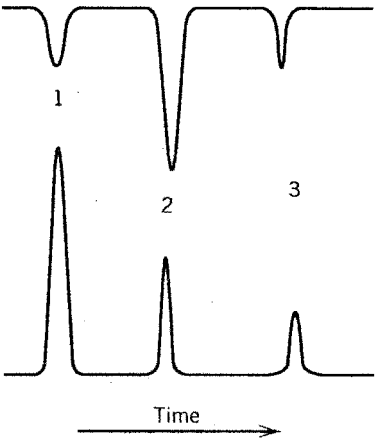

FIGURE 8.6 Mass chromatogram.

obtained for known compounds. The molecular weight and the Kovats retention index can then be combined to aid in the identification of the component. A linear relationship exists between the molecular weight and retention index for a homologous series of compounds. The relationship varies for each class of compound; thus a clue can be obtained regarding the type of compound present, which can be verified by some other technique.

8.2.6 Simple Pretreatment

A few minutes devoted to simple pretreatment of the sample can save many hours of interpretation of the complex data. Procedures such as filtration, extraction, or distillation can be readily accomplished and will simplify the identification of separated components (see Chapter 11).

8.2.6.1 Extractions

Simple partition phases can add another valuable piece of information about the sample. A gas chromatographic analysis before and after extraction indicates the character of the components present. For example, carboxylic acids are readily separated from the phenolic compounds by extracting a nonaqueous solution of the sample with dilute aqueous sodium bicarbonate. The carboxylic acids are almost completely transferred to the aqueous phase, whereas phenolic constituents remain in the organic layer. Additional information on extractants for specific classes can be found in most organic analysis textbooks or inferred from solubility tables. See Chapter 11 for more details of this technique.

8.2.6.2 Beroza's p Value

An additional mechanism of identification has been reported by Beroza and Bowman (10–13). The technique involves a measurement of the distribution of the unknown component between two immiscible liquids. This work was directed

primarily toward pesticide residue analysis. With reference to pesticide residue analysis, the sample processing invariably provides the pesticides in an extraction solvent of hexane or isooctane. Occasionally this may be another solvent. In any case, the prepared sample extract is chromatographed by the use of the appropriate instrumental sensitivity settings to generate a properly measurable chromatogram. A portion of the remainder of the prepared extract is then equilibrated with the same volume of an immiscible solvent, such as acetonitrile or an acetone–water mixture. Again a portion of the hexane or isooctane phase after equilibrium is chromatographed under precisely the same conditions. The peak height or area of the compound of concern is determined by both chromatograms. The ratio of signal following the equilibrium divided by the signal before equilibrium has been defined as the *p value*:

$$p \text{ value} = \frac{\text{peak height (or area) after partition}}{\text{peak height (or area) before partition}} \tag{8.4}$$

The p value for standards that have the same retention time under the same chromatographic conditions are then determined. The p value for the unknown component and one of the standards should be the same if the unknown and standard are the same compound. If two or more of the standards have closely similar p values and corresponding retention times, the experiment should be repeated using a different solvent pair.

For convenience, the p value was selected to designate the component distribution in solvent systems of equal volumes. If different volumes of the two solvent phases are used, appropriate corrections must be made (13). Ideally, the solvent systems should be chosen so that the p values for components of interest range between 0.25 and 0.75 to provide the greatest precision and assurance of identity or nonequivalence with the standard.

The p values for over 100 pesticides and related substances were established by studying the extraction behavior of those compounds in a wide range of binary solvent systems. As a result of these studies, Beroza and Bowman concluded the following:

1. Each pesticide exhibits a characteristic distribution ratio (p value).
2. Distribution ratios are practically independent of pesticide concentration over any range of concern in trace analysis.
3. Other components extracted from the original sample do not appreciably affect this ratio.
4. Compounds other than pesticides can be identified by the use of this p value and the gas chromatographic technique.

The use of distribution coefficients or their simplified equivalents as p values is not new and is based on sound chemical principles. Its particular value, however, is that it can be applied as a confirming means of identification where the component of concern is not available in sufficient quantity for the more common

analytical identification techniques, such as mass spectrometry, IR spectroscopy, elemental analysis, or physical property measurements (14). Its elegance rests in its simplicity.

8.2.6.3 Water–Air Equilibrium

McAuliffe (15) introduced a multiple-phase equilibrium procedure for the qualitative separation of hydrocarbons from water-soluble organic compounds. For *n*-alkanes, more than 99% was found to partition in the gas phase after two equilibriums with equal volumes of gas and aqueous solution. Cycloalkanes require three equilibriums to be essentially completely removed, and oxygen-containing organic compounds (e.g., alcohols, aldehydes, ketones, and acids) remain in the aqueous layer. After equilibrium with equal volumes of gas, an immediate clue is given regarding the identification of the compound. More details of this technique can be found in Chapter 11. This technique also provides two additional pieces of information: the distribution coefficient (D_s or D_g) and the initial concentration of the unknown component.

8.2.7 Tandem Gas Chromatographic Operations

8.2.7.1 Two Columns in Series

Some of the problems associated with obtaining a retention time or index on two different columns individually can be overcome by running two columns in series. A good example of this is the analysis of benzene in gasoline. On a methyl silicone column benzene will elute between *n*-hexane and *n*-heptane. Some major components of the gasoline also have retention times in this area such that the benzene would be completely swamped by these components. Similarly, a very polar column such as 1,2,3-tris(2-cyanoethoxy) propane (TCEP) will have benzene eluting between undecane and dodecane. There are sufficient hydrocarbons in gasoline in this range also that would obscure or make the confirmation of the presence of benzene extremely difficult. Thus, even though two columns were used, the presence of benzene certainly is not proved. If these same two columns were worked in series, however, the sample could be introduced into the silicone column and the effluent from that column directed into the TCEP column. Following the retention time of heptane on the silicone column, the higher hydrocarbons still remaining on the silicone column after heptane could be either backflushed or eluted forward through the column but not allowed to enter the TCEP column. A second carrier-gas flow would then elute the components from the TCEP column. In this case all the hydrocarbons up through heptane would emerge well before the benzene, and in this case the benzene peak would be isolated and completely identifiable. This technique of two columns in series should be considered when one is attempting to confirm the presence or absence of a specific component in a very complex mixture.

8.2.7.2 Subtractive Precolumns

For many applications the mixture to be analyzed is so complex that the only reasonable method of analysis requires the removal of certain classes of compound.

This process can be easily implemented by the use of a reactive precolumn. For example, a precolumn of potassium hydroxide can be used to remove acid vapors. The mixture could then be chromatographed with and without the precolumn to identify peaks of acid character. A discussion of precolumn reagents is given by Littlewood (16). Potential packing materials for precolumns may also be found in the trace analysis literature.

8.2.7.3 Carbon Skeleton

The technique of precolumn catalytic hydrogenation can be applied to reduce certain unsaturated compounds to their parent hydrocarbons. Other classes of compounds also analyzed by this technique include esters, ketones, aldehydes, amines, epoxides, nitriles, halides, sulfides, and fatty acids. Fatty acids usually give a hydrocarbon that is the next-lower homolog than the parent acid. For most systems utilizing hydrogenation, hydrogen is also used as the carrier gas. Usually 1% palladium or platinum on a nonadsorptive porous support such as Chromosorb P-AW is used as the catalytic packing material. This operation can be performed with two columns in series such that only a single component or a selected range of retention times of components from the first column is directed to the hydrogenation catalyst, which is then followed by a second column to observe the hydrogenated products of that particular segment. If one has a relatively pure material and is attempting identification, the injection can be made directly into the catalytic column followed by a column to identify the reduced hydrocarbon species.

8.2.7.4 Controlled Pyrolysis

The principle of controlled pyrolysis or controlled thermolytic dissociation for the identification of chromatographic effluents lies with the examination of the pattern ("fingerprint") produced. The peak selected for identification from the first column is transferred with continuous flow from the gas chromatograph through a gold coil reactor helically wound on a heated stainless-steel core, and then through a second gas chromatograph for identification of the pyrolysis products. The products are identified by comparing the Kovats retention indices to those of standard compounds and by enhancing the peaks with selected standard compounds. The fingerprint can also be obtained with increased certainty by coupling a mass spectrometer to the second chromatograph. The controlled pyrolysis technique can be especially useful in forensic (see Chapter 16) and toxicological applications when direct comparison is necessary. Information concerning functional groups absent or present in the molecules can be obtained by determining the concentration ratios of the small molecules produced on pyrolysis (CO, CO_2, CH_4, C_2H_4, C_2H_6, C_3H_6, NH_3, H_2S, and H_2O). "Large molecule" pyrograms (C_4H_8 and larger) in combination with "small molecule" pyrograms can give additional information regarding the functional groups present.

8.3 IDENTIFICATION BY GAS CHROMATOGRAPHIC AND OTHER DATA

An unequivocable identification of an unknown component is unlikely by the chromatographic process alone. Not the least of the reasons for this is the need for the comparisons of standards, thereby assuming reasonable prior assurance of the possible identity of the unknown. Certainly the more discrete pieces of information obtainable concerning an unknown compound, the easier it will be to obtain confident identification. Microchemical tests such as functional group classification, boiling point, elemental analysis, and derivative information, as well as infrared spectroscopy, coulometry, flame photometry, and ultraviolet (UV)–visible spectroscopy are also useful aids when used in conjunction with gas chromatographic data.

8.3.1 Elemental and Functional Group Analysis

The major reason why GC is not generally used for qualitative analysis is that it cannot differentiate or identify indisputably the structure of the molecule. Therefore, it is necessary to perform additional tests on the separated peak to ascertain its functionality and elemental composition. Many books and articles are available regarding microanalysis, so this method is not extensively reviewed here. Usually it is necessary to trap the peak, then perform whatever specific microanalysis techniques are necessary to confirm the identity of the peak. Several commercial instruments are available for elemental analysis (usually carbon, hydrogen, sulfur, and halogens), or by GC (see Reference 17). These instruments usually require 0.1–3 mg of sample and often employ trapping systems for quantitative analysis.

Hoff and Feit (l8) reacted samples in a 2-mL hypodermic syringe before injection onto the gas chromatographic column. Reagents were selected either to remove certain functional groups or to alter them to obtain different peaks. Reagents used included metallic sodium, ozone, hydrogen, sulfuric acid, hydroxylamine, sodium hydroxide (20%), sodium borohydride (15%), and potassium permanganate (concentrated).

A stream splitter attached to the exit tube of a thermal conductivity detector can be used to identify the functional groups of gas chromatographic effluents. Table 8.2 lists functional groups tests and the limits of detection. A review of elemental analysis is given by Rezl and Janak (19).

Crippen's excellent book (20) gives an extensive compilation of the techniques of organic compound identification with the assistance of GC. It includes a stepwise account of the preliminary examination, physical property measurements, and functional group classification tests. There are numerous graphs relating retention times with various physical properties such as melting point, boiling point, refractive index, and density. This book is a must for any extensive gas chromatographic laboratory dealing with unknown samples. Gas chromatographic methods for qualitative analysis of complex systems such as biological materials and bacteria proteins, steroids, and triglycerides also have been developed.

TABLE 8.2 Functional Group Classification Tests

Compound Type	Reagent	Type of Positive Test	Minimum Detectable Amount (μg)
Alcohols	$K_2Cr_2O_7$-HNO_3	Blue color	20
	Ceric nitrate	Amber color	100
Aldehydes	2,4-DNP	Yellow ppt.	20
	Schiff's	Pink color	50
Ketones	2,4-DNP	Yellow ppt.	20
Esters	Ferric hydroxamate	Red color	40
Mercaptans	Sodium nitroprusside	Red color	50
	Isatin	Green color	100
	Pb $(OAc)_2$	Yellow ppt.	100
Sulfides	Sodium nitroprusside	Red color	50
Disulfides	Sodium nitroprusside	Red color	50
	Isatin	Green color	100
Amines	Hinsberg	Orange color	100
	Sodium nitroprusside	Red color, 1[c] Blue color, 2[c]	50
Nitriles	Ferric hydroxamate-propylene glycol	Red color	40
Aromatics	HCHO-H_2SO_4	Red-wine color	20
Aliphatic unsaturation	HCHO-H_2SO_4	Red-wine color	40
Alkyl halide	Alc, $AgNO_3$	White ppt.	20

Source: Reprinted with permission from *Anal. Chem.* **32**, 1379 (1960).

8.3.2 Coupling Gas Chromatography and Other Instrumental Techniques

A technique that has become more common for the identification of compounds is the combination of gas chromatography and mass spectrometry (MS). This is due in part to the decreasing cost, increasing sensitivity, and decreasing scan time of mass spectrometry equipment. Read Chapter 7 for a complete discussion of this most important technique. Now it is possible to obtain not only a complete mass spectral scan of a gas chromatographic peak "on the fly," but also the mass spectra at various portions of the peak such as the front edge, the heart of the peak, and the tailing edge. This is especially useful in helping to ascertain whether a given peak is a single- or multiple-component peak, in addition to determining what those components are. This technique in general does not require a prior knowledge, or reasonable suspicion even, of the identity of the component to be identified. The most conclusive identification will be the recreation of the same mass spectrum from a known standard. The spectrum obtained from an unknown, if not immediately decipherable, will provide a significant number of clues to the

probable identity, thus limiting the need either for searching reference spectra or for the generation of a reference spectra.

As stated above, GC is unparalleled as a separation technique, but it lacks the ability to confirm peak identity from retention data only. MS is an excellent qualitative technique, providing confirmatory analysis guidelines to determine the presence or absence of a parent ion and two daughter ions in the appropriate proportions. What one obtains is the best of both worlds: good resolution by GC and molecular identity of the individual unknown peaks. Commercial spectral libraries (2l) make compounds identification easy. A word of caution: there are mistakes in computer based data. One may circumvent this problem by using multiple quadrupoles or ion trap analyzers that will provide the performance of MS/MS, providing additional structural information. GCMS sensitivity may be enhanced by selectively choosing a small set of ions characteristic of the analyte molecule (SIM mode). Detection limits can easily go down to 1.0 ppm in the scan mode, while ppb levels are achievable in the SIM mode.

There is really a question of semantics regarding the mass spectrometer coupled to a gas chromatograph. Most gas chromatographers would consider the mass spectrometer as simply another of several selective detectors available for use in helping to identify compounds. It would then be considered as entirely a gas chromatographic technique as opposed to GC coupled with other analytical techniques. The other side of the story is simply that mass spectroscopists would certainly consider the gas chromatograph as just another one of many inlet systems that they have available for their mass spectrometer.

The second most frequently used instrumental technique is infrared spectroscopy. In general, the first instrumental method to consider is the one most readily available. In a few cases, notably mass spectrometry, the technique may be used in tandem with the gas chromatograph, but in general most techniques require trapping of the peaks as discussed in Section 8.3.3.

Consideration must be given to the quantity of the sample needed for the minimum detection limits of the instrumental technique used. A number of techniques have been ranked in order of increasing amounts of material needed as follows: mass spectrometry (1–10 μg), chemical spot tests (1–100 μg), infrared and ultraviolet spectroscopy (10–200 μg), melting point (0.1–1 mg), elemental analysis (0.5–5 mg), boiling point (1–10 mg), functional group analysis (1–20 mg), and nuclear magnetic resonance spectroscopy (1–25 mg).

8.3.3 Trapping of Peaks

Trapping a sample as it elutes from the column followed by some other identification or classification technique is often utilized with gas chromatographic analysis. The most common trapping devices are the cold trap, the gas scrubber (gas-washing bottle), the evacuated bulb, and the absorbent postcolumn. A simple cold trap can be constructed from the small-diameter glass tubing, such as melting point capillary tubing, and connected with some flexible inert tubing to the outlet port of the chromatograph (Figure 8.7A). Part of the coil should be immersed in a liquid coolant such as liquid nitrogen (−196°C), dry ice–acetone

(−86°C), sodium chloride ice (1−2) (−21°C), or ice water slush (0°C). One should not use liquid nitrogen when air or oxygen is being used as the carrier gas because of the explosion hazard as liquid oxygen accumulates. The upper part of the coil should be above the coolant liquid so that loss of sample due to too rapid cooling (fogging) can be avoided.

A gas-washing bottle (Figure 8.7B) may also be used for trapping. This technique is especially useful in conjunction with infrared analysis. The sample is simply bubbled through the anhydrous solvent as it exits the chromatographic column. The solution is then placed in a liquid sample infrared cell. A matching cell containing only the solvent is placed in the reference beam. An infrared spectrum of the sample may then be recorded.

Evacuated bulbs (Figure 8.7C) are generally used for trapping volatile components. Since this technique does not concentrate the sample, additional sample preparation may be required. For substances with high infrared absorptivity, the sample may be trapped directly in an evacuated infrared gas cell and analyzed directly. For nonvolatile samples that may condense on the outside walls, the cells must be heated before analysis.

An adsorbent postcolumn can also be used to trap eluting peaks. Packing materials such as Tenax-GC (Enka N.V., The Netherlands), Porapak N and Porapak R (Waters Associates), Carbosieve B, and 20% DC-200 have been tested as sampling tubes for concentrating organic compounds in air. Tenax-GC and Porapak N seem to have the widest general applicability. Tenax-GC was more suitable for higher-boiling compounds, and Porapak N was more suitable for

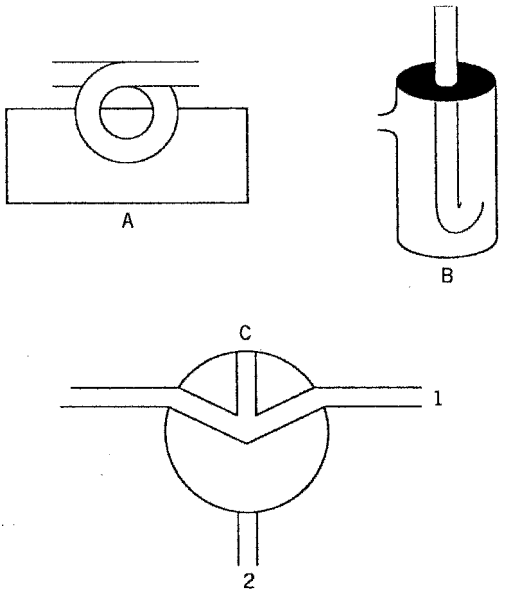

FIGURE 8.7 Traps: A, simple coil cold trap; B, gas-scrubbing trap; C, evacuated-bulb trap; 1, to outlet of gas chromatography; 2, to evacuated gas-sampling bulb.

lower-boiling organics (20–100°C). In many cases, in order to trap sufficient amounts of materials for subsequent analysis, many repetitive injections must be made into the chromatograph and more sophisticated trapping techniques may be required.

8.4 QUALITATIVE ANALYSIS WITHOUT PEAK IDENTIFICATION

There are many cases, especially in the analysis of very complex mixtures of materials, where identification of the source of material can be determined without the individual identification of any single chemical entity in the mixture. In these cases the chromatograms of the mixtures are simply used as somewhat of a "fingerprint" analysis. The general appearance of the peak, as far as retention times versus response is concerned, is the first piece of information, and in some cases ratios of peaks at given retention times can be used to facilitate the identification procedure. The power to resolve a complex mixture into its components and thus provide this "fingerprint" is an important technique of GC that should not be overlooked. There are many instances of the use of this technique. For instance, the origin of a particular spice can be determined by comparing its "fingerprint" chromatogram against the fingerprint of a sample known to be genuine. Paint chips can be compared by using a pyrolysis technique ahead of the gas chromatograph. In this particular instance the pyrolysis products of the paint chip are used as a "fingerprint" and in many cases can be used to identify the source of a given paint chip. Two other techniques where this "fingerprinting" of the gas chromatographic printout has been used to advantage are determining arson accelerants (Chapter 16) and oil spills (Chapter l5)

8.5 LOGIC OF QUALITATIVE ANALYSIS

The most important factor in qualitative gas chromatographic analysis is the collection of as much information as possible about the sample before beginning any laboratory work. This information is first gathered by the people involved in the collection of the sample. The sampling location, the person taking the sample, the method of sampling, and sample handling should be known. The sample matrix (solvent, etc.) should be investigated to determine the source of chromatographic peaks. A pure sample should be utilized to compare with the unknown sample. The technique of running blanks on solvents should certainly not be overlooked since the solvent used to work up a sample may be the contributing factor to unknown peaks. Furthermore, the chemist should always be alert to unknown peaks originating from simple decomposition in storage or decomposition or isomerization under chromatographic conditions. All of the above items are important considerations, especially in the area of trace analysis. Many times impurities can be in excess of the amount of trace components being analyzed. One should keep in mind that the identification of an unknown by GC can easily turn into a major research project.

PART 2 QUANTITATIVE ANALYSIS

8.6 GENERAL DISCUSSION

The gas chromatographic technique is at best a mediocre tool for qualitative analysis. As has been shown previously, it is best used with other techniques to answer the question of what is present in the sample. The rapid growth of GC since the early 1950s cannot be explained by ease of operation, the simplicity of the technique, the relative low cost of the instrument, or the wide range of the types of samples being handled. That growth comes from the fact that GC has all these attributes and provides an answer to the question "How much?" Its reason for existence is that it is an excellent quantitative analytical tool regardless of whether one is quantifying micrograms of heptachlor in a liter of water or one volume carbon monoxide in a million volumes of air.

Sometimes we get carried away with the latest advancement in instrumentation or with the perfectly symmetric peaks obtained with a certain system. These are only means to an end, perhaps very necessary means, but they are not the end. The end is a number that tells us how much of a component is in a sample. Without the ability of GC to supply that number with reasonable accuracy, this entire book would not be written. The tremendous advances in instrumentation, theory, columns, applications, and technique are all justified because they provide more accurate and precise analyses, analyses for materials not previously possible, or much more rapid analysis.

The remainder of this chapter deals with the techniques used to obtain the answer to the question "How much?" from the information given by the chromatograph. The quantitative principle of GC depends on the fact that the size of the chromatographic peak is proportional to the amount of material. The first aspect to be considered is the technique of determining peak size. Next, the problem of relating peak size to quantity of material is discussed. Finally, factors that influence peak size and thus introduce errors are considered.

8.7 PEAK SIZE MEASUREMENT

The size of a chromatographic peak is proportional to the amount of material contributing to that peak, and the size of this peak can be measured by a number of ways. Each of these is considered individually. Two basic concepts can be used for peak size. The first is simply the measurement of the height of the peak. The second involves the measurement of area with a wide variety of methods available. Each technique when used properly is an acceptable means for quantitation.

8.7.1 Peak Height

Peak height is the simplest and easiest of the measurement techniques. As shown in Figure 8.8, the baseline is drawn by connecting the baseline segments both

before and after the peak (line *AB* in the diagram). This line would be the best estimate of the detector output if there had been no detectable amount of material present that contributed to that peak. The height of the peak is then measured from this baseline vertically to the peak maximum (line *CD*). This height is proportional to the amount of material contributing to the peak if nothing in the system changes that could cause a change in the width of the peak between sample and standard.

Factors that can influence the peak width are generally instrumental or technical in nature. The temperature of the column changes the retention time of the material, thus changing the width of the peak. To a first approximation the ratio of retention time to peak width will stay constant for a given component on a given column. Temperature can influence retention time by approximately 3%/°C. A 1°C change in column temperature between the standard and the unknown chromatograms can cause a 3% change in peak width. This change in width will be accompanied by a compensating change in peak height such that height times width remains constant. The height then will change by 3%/°C. This means that to maintain analysis at 1% accuracy by use of peak height measurement, the temperature of the column must be controlled within ±0.3°C and preferably to better than ±0.1°C, assuming the temperature change of the column to be the only factor producing error. Detector temperature may also affect the peak height measurement since the detector's response may be temperature-sensitive. This problem will affect any measurement of peak size generally in an indetermined way. Thus, excellent control of detector temperature is also important in any quantitative use of a gas chromatograph.

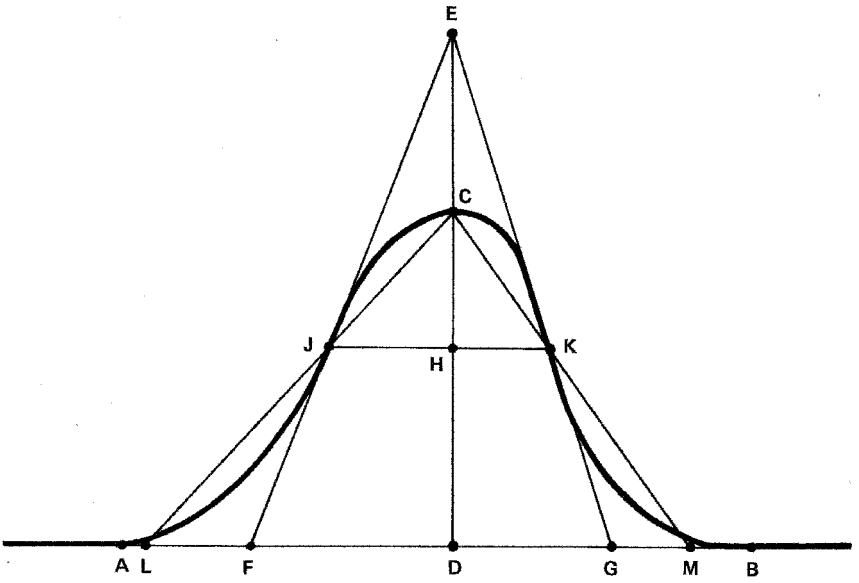

FIGURE 8.8 Constructions for peak size measurements.

The carrier-gas flow also produces a change of retention time and thus peak width and peak height. To a first approximation, a 1% change in flow will change retention time 1%; thus peak height and peak width are changed by 1%. Use of peak height measurement thus requires that the flowrate between the standard and the sample chromatograms be constant within 1% to maintain an accuracy of 1%. The previously mentioned consideration regarding the effect of flow on the error of peak height measurement is independent of the major error consideration regarding constant flow. Several detectors, notably thermal conductivity and photoionization, are flow-sensitive; that is, the sensitivity or electrical output for a given amount of material varies with flow. This flow effect affects any method of peak measurement and is really not an error of size measurement. It simply says that good flow control is needed regardless of the method of peak measurement.

Reproducibility of peak height is also quite dependent on the reproducibility of the sample injection. This is especially important on early, and thus normally quite sharp, narrow peaks. On such early peaks, the width of the peak is controlled more by the injection time rather than the chromatographic process. A fraction-of-a-second increase in injection time can double the width of these peaks and reduce peak height by 50%. The peaks most subject to error in peak height measurement from injection problems are those with retention volumes 1–2 times the holdup volume V_m of the column. Peaks beyond 5–10 times the holdup volume are negligibly affected by injection technique.

Automatic injection systems can reduce the variability in the precision of the injected volume. Since autoinjection systems are more reproducible than manual injections, autoinjection should be used, where possible, for all quantitative measurements.

When there is column adsorption of a particular component in the system, the peak will show some tailing. This may not be evident at high concentrations of a component, but with low concentrations a significant portion of the component may be in the tail. This means that at low concentrations the relationship of peak height to amount of materials may not be linear because of the amount of the material in the tail. For quantitative analysis, it is best to avoid adsorption by a better choice of column regardless of the technique of peak measurement. However, with adsorption, peak height may give a significant error with low amounts of material.

The final consideration of peak height measurement is the phenomenon of column overload. When a large amount of a component is injected onto a chromatographic column, the liquid or adsorptive phase becomes saturated with the material, causing a broadening of the peak. This causes reduction in the height, contributing to a nonlinear relationship between peak height and amount of material with high amounts of material. This is independent of any detector nonlinearity at high concentrations. Overloading can be observed by careful observation of the peak shape. There is sloping front edge with a sharp tail, or in some cases, a sharp front with a sloping tail. The peak maximum also moves with this distortion to longer times with the sloping front and to shorter times with

the sloping tail (see Chapter 2 and Section 8.2.1). This overload distortion is a function of the amount of liquid phase per unit length of column. It occurs more readily on small-diameter columns and on packed columns with a low-percentage of liquid phase.

8.7.2 Height and Width at Half-Height

Contrary to peak height measurement, a number of techniques are used for peak area measurement. Some of these are manual techniques, and others make use of instrumental accessories to provide an area measurement. The discussion that follows considers all of these techniques from the manual through the instrumental, in that order. Although most chromatographers use peak area data from computer systems or electronic integrators, manual and instrumental methods are included here to both enhance the understanding of the concept and allow chromatographers to re-create quantitative data from old data for litigation or patent purposes.

In height–width measurement the area is determined by multiplying the height of the peak by the width of the peak at one-half the height. This technique requires the construction of the baseline (line *AB* in Figure 8.8) and the measurement of the height of the peak *CD* as in the peak height technique. Point *H* is then determined as being halfway between points *C* and *D* such that *DH* is one-half the height *CD*. Line *JK* is then drawn parallel to *AB* and through *H*. The distance *JK* is thus the peak width at half-height. The product of *CD* and *JK* is the exact area of the triangle *CLM*. It is a close approximation of the true area of the chromatographic peak. It includes an area below the line *JL* that is not a part of the peak, but excludes some peak area above the line *JC* that is a part of the peak. To the extent that these areas compensate each other, the area of triangle *CLM* is equal to the area of the chromatographic peak.

If the baseline is sloping for any reason, the measurement becomes a bit more complicated. Figure 8.9 is constructed as Figure 8.8, with the same parts of the construction labeled with the same letters. The baseline *AB* is constructed as the best extension of the baseline before and after the peak. The peak height *CD* is constructed vertically from the peak maximum to the baseline. The midpoint *H* is located as before. Line *JK* is then drawn through point *H* and parallel to the baseline *AB*. The desired peak height is the distance *CD*. However, the width at half-height is then the distance *NP*. This would be the width measured with no slope in the baseline. Note that the distance *JK* could be used if it were corrected by the cosine of the angle *JHN* or the angle of the baseline to a true horizontal. The important point here is that points *J* and *K* are the true points on the peak one-half the height of the peak up from the baseline. What is wanted for the width measurement then is the real-time separation of points *J* and *K*, which is given by the horizontal component only of the distance between them.

The various errors of this technique are summarized at the end of this section for all area techniques.

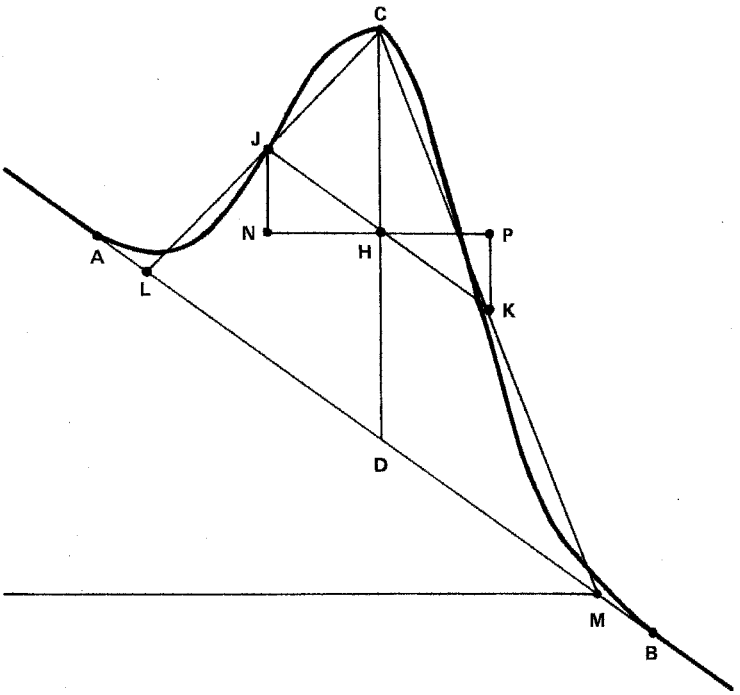

FIGURE 8.9 Constructions for peak size measurements on sloping baseline.

8.7.3 Triangulation

Triangulation always involves construction of the baseline *AB*. Tangents to the peak are then drawn at the inflection points of the peak. These tangents are lines *EF* and *EG* in Figure 8.8 and, along with the baseline, form the triangle *EFG*. The area of this triangle is the height *ED* times one-half the base *FG*. This area closely approximates the area of the peak. Comparison of the various area techniques, presented later, includes a discussion of the problem with this technique. For this reason a more detailed discussion here regarding sloping baselines is not warranted.

8.7.4 Cut and Weigh

This technique, sometimes referred to as "paper dolls," involves drawing of the baseline *AB* as before. Then the peak is carefully cut out of the chart paper and weighed on an analytical balance. This weight is then converted to an area by weighing a known area cut from the same chart near the peak. The major advantage of the technique is that it can accommodate distorted and tailing peaks, giving a true measure of the area. The major problem is the inhomogeneity of the paper and the destruction of the chromatogram. Certainly copies can be made

prior to cutting, or the copy itself could be cut. The general errors associated with this technique are reserved for comparison at the end of this section.

8.7.5 Planimeter

Like the cut-and-weigh method, the planimeter method is a perimeter method that makes use of a surveying or drafting instrument called a *planimeter*. In this technique the baseline is drawn as usual. The perimeter of the peak is then traced using the eyepiece containing crosshairs of the planimeter. When the starting point is reached, the dial reads a number proportional to the area. On some planimeters the number is proportional to area with application of a settable scale factor. On other instruments the factor must be determined by measuring a known area. The major advantage of the technique is the ability to handle distorted and tailing peaks to produce a true area. The major problems are the painstaking nature of tracing the peak and the use of a tool not normally found in a laboratory.

8.7.6 Disk Integrator

The simple ball–disk integrator (briefly described here for historical reasons) attaches to the recorder. The integrator pen draws a trace on about 10% of the chart, leaving 90% for chromatogram as drawn by the recorder pen. The integrator pen is linked mechanically to the ball through the cam and roller, and the ball rides on the disk that rotates at a fixed speed. When the recorder pen deflects the ball (which is linked to the recorder pen drive), it moves away from the center of the disk and begins to rotate at a speed proportional to its distance from the center. The roller begins to rotate at the speed of rotation of the ball. The cam then causes the integrator pen to oscillate on the chart at a rate proportional to the ball rotation. Thus the number of integrator pen excursions between the beginning and the end of the chromatographic peak is directly proportional to the peak area. A single excursion is assigned a value of 100 counts. A partial excursion generally can be estimated to ± 1 count.

8.7.7 Electronic Integrators and Computers

In general, electronic integrators are fed the detector signal directly without attenuation. Following amplification, this voltage signal may be converted to a frequency such that the output pulse rate is proportional to voltage and the pulse sum is proportional to area. Generally, however, with microprocessor-based integrators the amplified voltage signal is simply sampled several times (2–10) per second, and the voltages are then summed to produce a number proportional to the area. A slope detector in either case detects when the peak begins and ends.

The major advantages of the electronic integrator are the speed and accuracy with which the area is obtained. These devices operate on the detector signal only and thus are limited only by the detector. Their wide dynamic range permits the integration of both trace and major components without attenuation. The high

count rate and sensitive voltage detection ensure accuracy well beyond any other mode of peak measurement. Most integrators can store calibration data and report areas or peak heights, retention times, and the final concentration of one or more components in the sample, using any of the techniques discussed in Section 8.8.

Most gas chromatographs have built-in integration and data-handling capabilities. Also common are networked systems to whereby a number of gas chromatographic detectors' signals are fed into a central computer for peak area measurement and data reduction. The advantage of a single computer is that the same software can be used to process the chromatographic information from many different chromatographs, thus eliminating one source of variability. The disadvantage of a network computer is that if something goes wrong with that computer, all chromatographs attached to the network are impacted.

The dedicated computer and its chromatographic software are powerful assets to the chromatographer. The algorithms associated with peak detection and area measurement can mimic human logic and expedite the data processing steps. But these same tools can also cause considerable frustration when they do not seem to mimic human logic. It is important that the human being associated with the instrument spend as much time as possible understanding how the software and hardware work and how to troubleshoot the system when they do not. Most vendors offer traditional and computer-assisted courses to help the chromatographer understand how to operate specific systems so that reliable data are produced. Many scientific groups also offer helpful courses given by experts in the field. Investing time in this training will pay great dividends. The discussion presented in Chapter 4 ("Optimization of Separations and Computer Assistance") should be read for a more thorough presentation. For further detailed information, the manuals, application notes, and product bulletins provided by the instrument manufacturers should be consulted.

8.7.8 Comparison of Peak Size Measurements

In 1966 a survey of over 1600 practicing gas chromatographers in the United States was made and reported by Gill and Habgood (22) on measurement

TABLE 8.3 Peak Size Technique in Use in 1966

Technique	Relative Usage (%)
Peak height	28.0
Triangulation	16.9
Planimeter	15.5
Cut and weigh	6.4
Disk integrators	20.8
Digital electronic integrators	8.5
Computers	2.4
Tape systems	1.4

Source: Data of Gill and Habgood (22).

techniques then in use. These are reported in Table 8.3. The large number of respondents would also tend to support the validity of the data. Speculation as to how this has changed over the years since the survey would lead to the conclusion that direct integration techniques of electronic integrators and computers have increased. Personal surveys of today's chromatographers would indicate that more than 90% use electronic methods in quantitative analysis.

Ball et al. (23–25) in a series of papers have considered the various manual techniques for the peak size measurements. The treatment was both theoretical and experimental in that standard peaks were given to a sizable group for manual measurement. Five techniques were studied: peak height, height and width at half-height, triangulation, planimetry, and cut-and-weight measurement. The actual measurement errors are summarized in Table 8.4. Peak shape is defined as the peak height divided by the peak width at half-height. In manual methods of peak measurement, accuracy, and precision of measurement degrade considerably as the peak shape becomes extreme. This occurs with very sharp peaks (peak shape >10) or very flat peaks (peak shape >0.5). Thus there is an optimum peak shape that the chromatographer should strive to achieve. In all cases of measurement, the greater the area, the better is the precision of measurement. In the case of peak height, the narrower the peak for a given area, the better is the precision. In all cases it is best to display the peak at the minimum attenuation (maximum sensitivity) and still maintain the peak on scale. For manual area measurements the chart speed should then be selected to give an optimum peak shape between 1.0 and 10.

It should be stressed here that the errors mentioned in Table 8.4 are for the measurement technique only. They do not represent the precision expected of the analysis.

Condal-Busch (26) has pointed out that triangulation gives 97% of the true area of a Gaussian peak, whereas height and width at half-height give only 90% of the true area. Since chromatographic peaks are not truly Gaussian, however, the error is less. Condal-Busch also concludes that since standards and unknowns are measured in the same way, this error becomes insignificant compared to the actual measurement error itself.

TABLE 8.4 Conditions for Least Error in Peak Size Measurements

Measurement Technique	Relative Least Error (%)		Peak Shape for Least Error[a]
	1.5-cm^2 Area	15-cm^2 Area	
Peak height	<1	<0.5	<1
Height × width at half-height	2	0.5	5
Triangulation	3.5	1.5	1
Planimeter	4	0.6	1–10
Cut and weight	3.2	2	1–10

[a] Peak shape is defined as peak height/width at half-weight.

McNair and Bonelli (27) report a study made comparing a number of techniques for area measurement wherein the entire chromatographic system was analyzed. An eight-component sample was used. The relative standard deviation of 10 replicate analyses by the different techniques is recorded in Table 8.5. In general, the data in Tables 8.4 and 8.5 are consistent if one remembers that Table 8.4 contains data on measurement technique precision only and Table 8.5 has data on the entire system precision.

The problem of peak measurement on a sloping baseline must be considered. For peak height, planimeter, and cut–weigh techniques, this is only a problem of drawing the proper baseline under the peak. As discussed previously, it is more complex for height–width and ball–disk techniques. In the case of electronic integration, severe error may be introduced or accurate correction may be made simply depending on the features and capability of the particular integrator. Errors of this type and their solutions have been discussed in detail (28–32).

Computer-based systems are by far the most common but not necessarily the most accurate. They have the advantage of focusing on any part of the baseline so that the chromatographer can see how the peak is integrated. The integration parameters can easily be adjusted to produce more (or less) accurate or precise data. The computer-based systems have both hard-drive and external memory for storing data. Since there are so many ways that these data can be manipulated, great care must be taken to ensure the same parameters are used in a sample set or in a continuing analysis.

In evaluating all of these observations and current practices, the following conclusions regarding peak size measurements seem evident:

1. For time saved and accuracy, electronic or computer integration is the preferred approach. In general, an integrator capable of handling drifting baseline and fused peaks accurately, although more expensive, is required.
2. The ball–disk integrator is capable of excellent results on all but the most exacting analyses. The recorder used with it must be of top quality and in excellent working order to obtain full capability of the integrator.
3. Peak height, because of its simplicity, speed, and inherent measurement precision, is the preferred manual method. Chromatographic conditions are

TABLE 8.5 Precision of Area Measurement

Measurement Technique	Relative Standard Deviation (%)
Planimeter	4.06
Triangulation	4.06
Height × width at half-height	2.58
Cut and weigh	1.74
Ball and disk	1.29
Electronic	0.44

Source: Data of McNair and Bonelli (27).

much more critical here than in any other measurement technique. Current instrumentation helps in this regard. However, more frequent standardization is the real solution.

4. The time required and the difficulty of accurate tangent construction makes triangulation a method that cannot be recommended under any circumstance.
5. Height–width is the preferred manual area technique assuming reasonable peak shapes.
6. Perimeter methods should be used on irregularly shaped peaks.
7. The cut–weigh method is quite time-consuming. However, with adequate control of variable paper density, it has real value for irregularly shaped peaks.

8.8 STANDARDIZATION

8.8.1 General

With techniques of peak measurement in hand, the next important step in quantitative analysis is to convert the size of the peak into some measure of the quantity of the particular material of interest. In some fashion this involves chromatographing known amounts of the materials to be analyzed and measuring their peak sizes. Then, depending on the technique to be used, the composition of the unknown is determined by relating the unknown peaks to be known amounts through peak size.

There is always the question of standards (known amounts of material generally in a matrix) regarding their preparation in the laboratory versus the purchase of readymade standards. In general, standards should be as close to the unknown samples as possible not only in the amounts of the materials to be analyzed, but also in the matrix of the sample itself. In all cases this requirement would dictate the preparation of standards in the laboratory. There is also the question of stability of the standards. With elapsed time, loss of either the matrix (e.g., hexane evaporation from a solution of pesticides in hexane) or the components of interest (e.g., adsorption of xylene on container walls of 50-ppm standard of xylene in air) cause the standard to be unreliable. In general, in the absence of prior knowledge, this dictates that standards be prepared, used, and then discarded all within a short period of time.

Generally, it is much easier to purchase gas standards already prepared and analyzed. Experience here would indicate that these standards be viewed skeptically until credibility has been established for a given source. Certainly rather specialized equipment is needed to prepare a gas mixture with known concentrations of components, but in some cases this is the only reliable way to obtain standards.

The question of purity arises regarding materials used to prepare standards. Two problems occur here: the purity of the component of interest and the purity

of the matrix. Fortunately, GC can be used to check purity of chemicals in a reasonable fashion, If a small (1-μL) sample of a "pure" liquid is injected into a chromatograph and the detector system is operated at reasonably high sensitivity, impurities will be observed. Without even identifying these impurities, it is generally possible to make some comment on the purity of the chemical relative to its use in a standard. This does not require the use of a general-type detector (e.g., thermal conductivity) rather than any of the specific detectors. If no impurities are observed where one might be expected to see approximately 0.05% of most materials, it is reasonable to assume that the purity is better than 99.5%. This reasoning could certainly be used to prepare a standard well within ±1% accuracy, assuming no other problems. There are a number of loopholes in this approach. Certainly the column system is overloaded for 1 μL of essentially a pure component. This causes the major peak to broaden and possibly to obscure an impurity very close to the major peak. In general, suspected impurities will be close to the major constituent as a result of similarities in chemical properties and boiling points. The advantage of checking compounds to be used as standards by chromatography is that contamination can be detected. This contamination may have been introduced by previous users of the chemical not using good analytical technique, the inadvertent use of unclean containers, and possibly by mislabeling.

Purchased standards should come with a certificate of analysis (COA). The COA must also be critically reviewed to understand what method was used for the standard's analysis. For example, if the purity of the standard was determined by isothermal GCMS, could higher-boiling components be present but unseen in the analysis chromatogram? If a nonchromatographic technique were used, could it have missed a component or measured a similar one incorrectly?

In general, one cannot be too critical regarding standard purity. However, a realistic approach must be taken. If an analysis is required at the 10-ppm level to ±1 ppm (10% relative), it is not reasonable to spend time and money obtaining standards with reliability to better than 1% when perhaps even 5% would be sufficient.

A reasonable approach to any standard preparation is to obtain the best accuracy in the standard that one can obtain quickly, and then see whether this accuracy will be the limiting factor in the final analysis. If it is limiting, however, further work is needed to improve the accuracy of the standard. In this light the separating power of GC should not be overlooked. The trapping techniques discussed for qualitative analysis by other techniques can be used to isolate small amounts of pure material for standard preparation.

With this introduction to standardization, three techniques are now discussed and the use of standards is covered in each technique.

8.8.2 External Standardization

The technique of external standardization involves the preparation of standards at the same levels of concentration as the unknowns in the same matrix as the unknowns. These standards are then run chromatographically under ideal conditions as the sample. A direct relationship between peak size and composition

of one or more components can then be established, and the unknowns can be compared graphically or mathematically to the standards for analysis.

This technique allows the analysis of only one or several components in the same sample. Standards can be prepared with all components of interest in each standard, and the range of composition of the standards should cover the entire range expected in the unknowns. The peak size is then plotted against either absolute amounts of each component or its concentration in the matrix, generally the latter.

Figure 8.10 shows a typical calibration curve for four methyl ketones in an air matrix in which peak heights were used as the size measurement. Note that at some of the higher concentrations, the actual chromatograms were obtained at sensitivity settings different from those at the lower concentrations.

Five separate standards were used to prepare Figure 8.10, and all four components were present in each standard. Two different calibration scales were used to separate the curves for ease of identification. Two very important items can be learned from the calibration curve. In general (and in Figure 8.10), the curves are straight lines, and they pass through the origin. These two requirements are most important, for they determine that under the conditions of analysis and over the concentration range covered (1) the column has not been overloaded, (2) the detector has not been overloaded, (3) the electronics are responding linearly, and (4) there is no apparent component adsorption in the injection port, the column,

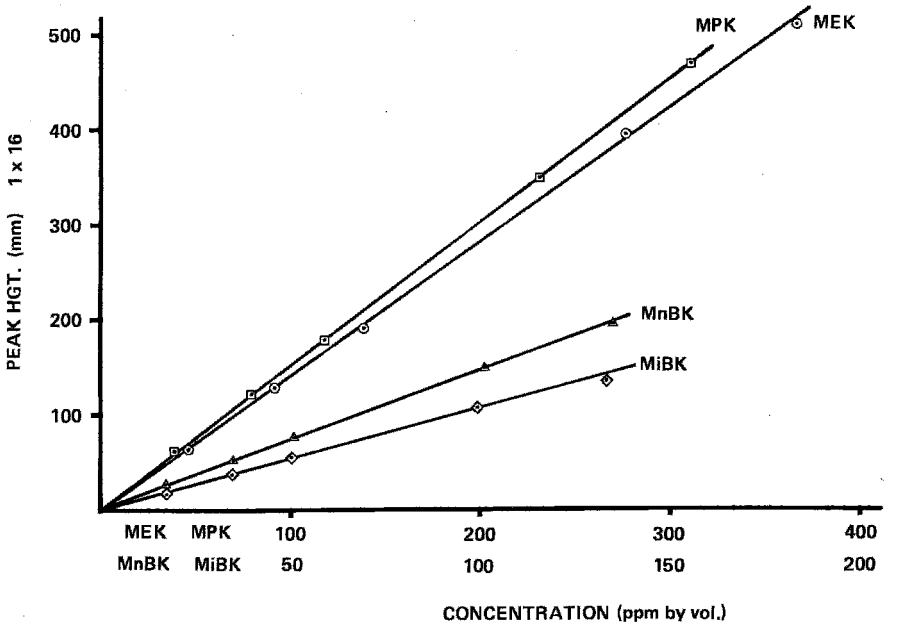

FIGURE 8.10 Calibration curves for four methyl ketones in an air matrix: 2-butanone (MEK), 2-petanone (MPK), 2-hexanone (MnBK), 2-methyl,4-pentanone (MiBK).

the detector, and associated plumbing. Note also that although one would expect that a sample blank (with no component of interest deliberately present) should give a low or zero signal when none is injected, the 0,0 point is not plotted. Points used for the calibration curve should come solely from the standards. These standards must bracket the expected concentration of the analyte.

At some point in any system, as the amount of component doubles, the peak size will not quite double. The column may overload, distorting peak shape; the detector capacity may be exceeded; or some other phenomena may occur. Where possible, one should operate below this point by using a smaller sample size or by diluting the sample. Although it is possible to perform quantitative analysis in a region where the system is nonlinear, this requires that the calibration curve be very well defined in the nonlinear region, meaning a large number of standards. It also means that the calibration curve must be redefined each time unknowns are analyzed. This obviously is quite time-consuming and should be avoided if at all possible.

Adsorption problems and/or sample degradation are generally the cause of failure of the calibration curve to extrapolate through the origin. These complications can often be avoided by proper sample handling and by proper choice of columns, both materials of construction and packing. It is possible to work with a calibration curve that does not pass through the origin, but this also requires that the calibration curves be generated quite frequently.

It is generally possible to obtain calibration curves as in Figure 8.10, where the concentration region of interest is linear and where the plot extrapolates through the origin. When one is satisfied that these two conditions are indeed met in a given analytical system, it is not necessary to regenerate these curves frequently by running various concentrations of standards. Slight changes of flowrates and temperatures of the detector and column may change the sensitivity of the system and perhaps even the response relationship between various components in the sample, but they will not change the linearity and the origin situation. For day-to-day calibration of the same system, one should run at least one standard and ratio concentrations and peak size for each component of interest between the standard and the unknown. What is involved, in effect, in this approach is recalculation of the slope of the calibration curve with a new standard. With any new system or any new analysis, however, the two basic requirements should again be verified by running several concentrations and plotting the calibration curve.

In regulated environments, the criteria for calibration are more rigorous. In addition to running a calibration set before the samples are run, the chromatographer might also have to either intersperse standards among the samples or rerun the standard set again at the end of the sample analysis. In cases where two or more sets of standards are run, criteria should be in place to assure that the data for the last unknowns are as reliable as the first unknowns run.

The major problem with external standardization is that the sample size of the standards and unknowns be known accurately. One should attempt to make them equal so that the size of the standard and the size of the unknown divides out of the calculation. If the sample size varies slightly, the peak size must

be corrected to unit sample size for standards before the calibration curve is plotted and for unknowns before the calculation is made. Sample size obviously enters into the calculations. As stated earlier, reproduction and measurement of sample size constitute the most critical single error in quantitative analysis by GC. Considerable attention is given to this technique of sample injection later in the discussion of general errors. It should be noted that, in the generation of calibration curves, it is absolutely unacceptable to vary the amount of the component injected by varying the amount of a single standard rather than by using the same amount of different standards having different concentrations. There is no doubt that doubling of the sample size results in doubling of the absolute amounts of each component injected into the chromatograph. But there is no guarantee that the chromatographic system will double the response obtained in the presence of double the amount of matrix. Sample sizes for all standards and unknowns should be kept the same within the errors of size measurement.

Because of the ease of reproducing injection volumes of gas with a gas-sampling valve and the difficulty of applying the technique of internal standardization discussed in the next section for gas samples, external standardization is the preferred approach for the analysis of gas samples. For these reasons considerable attention is given to the preparation of gas standards and the problems associated with gas analysis. In many cases this touches also on the area of trace analysis, since much of the gas analysis done today is the analysis of trace components in an air matrix.

8.8.2.1 Static Gas Standards

All static methods involve mixing of known amounts of gases or vapors together in some form of a container. These amounts may be measured by volumes or pressures, depending on the types of equipment available. Mixes of the permanent gases in the percentage range are generally reliable. However, they should not be used as primary standards without verification and prior experience (33). These mixtures are generally analyzed and thus become secondary standards.

Difficulties are encountered with these mixtures as the concentration of some of the components approaches the 1–100 ppm (v/v) range. Reaction and adsorption become problems even for gases normally considered fairly nonreactive. One report (34) of two CO standards certified at 26 and 41 ppm by the same supplier gave 51 ppm for the second one (25% error) with the instrument calibrated using the first one. Two conclusions arise from this: (1) at least one "certified" standard is wrong, and (2) even "certified" standards should not be trusted implicitly without verification.

Pretreated cylinders with proprietary coatings or treatments have shown some promise of overcoming reaction and adsorption of even some reaction gases. Even assuming that a mixture stays constant in such cylinders, the true concentration must still be known. If the mixture does not remain constant, the situation is impossible.

With the use of compressed gas standards, extreme care is needed in the hardware used between the cylinder and the injection of the standard into the

chromatograph. In many cases the cylinder supplier can recommend the proper valving and regulators to use. The issue here is not merely what is safe but also what will not add to or subtract from the standard gas passing through it. In some cases valves rather than regulators should be chosen. For the sake of safety, one should not rely on the cylinder valve for control.

Standards are available today in small pressurized cans that are extremely convenient to use with a gas-sampling valve for injection. Again, supplier reliability and verification are a must.

Laboratory preparation of standard mixtures can be made. In general, the static methods are used only for low concentrations in a matrix gas. Fixed-volume containers made from inert materials, capable of being sealed, and having a resealable septum system can be used. One-gallon glass jugs with lids modified for a septum are very common. On small containers the volume can be determined by weighing the container before and after filling with water and then converting the weight of water to a volume. In some cases quite large containers are used, and here the volume is generally calculated from measured dimensions. In either case some means must be provided to facilitate the mixing of the mixture to provide homogeneity. Diffusion is not sufficient. In small containers this can be a piece of heavy-gauge aluminum foil that can be shaken in the container. In large containers it is generally a fan blade or a blower. The container is thoroughly flushed with the matrix gas until it is reasonable to assume that the container has matrix gas only. It is then sealed and a small volume is withdrawn through the septum for analysis by GC. This is to ensure that the matrix in the container is free of the component to be added to within the error of the needed standard. Failure to perform this simple check can result in many problems and wasted effort.

For gases, a gastight syringe is flushed thoroughly with the component to be added, filled with the needed amount of pure component, and then emptied into the container through the septum. The concentration is simply a ratio of the volumes

$$\%\text{A} = \frac{\text{volume A added}}{\text{container volume}} \times 100 \tag{8.5}$$

or

$$\text{ppm A} = \frac{\text{volume A added}}{\text{container volume}} \times 10^6 \tag{8.6}$$

The concentrations are volume or mole percent or parts per million (v/v). This is the usual method of presenting gas concentrations, as opposed to those on a weight basis. The container must be thoroughly mixed to ensure homogeneity. The two major sources of error of this technique result from inadequate mixing and lack of assurance regarding whether the syringe volume used contained 100% of the desired component. One never knows when both conditions have been satisfied; therefore overcaution is the word.

Known concentrations of vapors can be prepared in the same way by injecting a known volume of a volatile liquid into the container, using a microliter syringe

normally used for liquid sample injection into a gas chromatograph. The density and molecular weight of the component are needed for the calculation:

$$\text{ppm A} = \frac{\text{volume A} \times \text{density} \times 24.25 \times 10^6}{\text{MW}_\text{A} \times \text{container volume}} \tag{8.7}$$

where volume A = μL of A added as a liquid
density A = density of A (g/mL or mg/μL)
24.45 = molar volume at 25°C and 760 Torr (101 kPa) (L/mol or mL/mmol)
MW = molecular weight of A (g/mol or A mg/mmol)
container volume = volume of container (mL)

The liquid syringe must be touched against the side of the container or the foil to obtain the final amount of injected liquid off the needle prior to its withdrawal from the container. The contents of the container must again be thoroughly mixed to ensure both complete evaporation of the liquid and a homogeneous mixture. If the temperature and absolute pressure of the matrix gas in the container differ from 25°C and 760 Torr (101 kPa) (the conditions of molar volume used), then either the container volume must be corrected to these conditions or the molar volume must be corrected to the conditions of the matrix gas. Differences of 3°C or 7 Torr (0.9 kPa) cause a 1% error. Generally, larger differences should be corrected. The important point to remember is that the volume of the vapor (calculated in Equation 8.7 by applying data for liquid volume, density, molecular weight, and molar volume) must be at the same temperature and pressure as the matrix gas for calculation of a volume ratio such as volume percent or volume part per million.

Several gases or vapors may be added to the container by either technique to provide standards for a number of components. A disadvantage of a fixed-volume container is that the sample is depleted as withdrawals are made. Generally, about 10 mL would be withdrawn to adequately flush a 1 mL volume of gas-sampling valve. Two such withdrawals will deplete a 2-L container by 1%. This depletion will cause a dilution of the standard by air, either from small leaks in the container or as the syringe is withdrawn from the sample under reduced pressure. Adsorption with time can be a serious problem, especially with vapors. Generally, the best practice is to prepare the standard using intermittent mixing over a period of 15–30 min. Then the chemist should use the standard, perhaps in duplicate or triplicate, and discard the standard. Unless experience has indicated a longer period of stability for a given system, these static standards should be trusted no longer than 1 h.

Plastic bags have been used to overcome the problem of fixed volume (35–37). However, other problems are introduced. The volume of the matrix gas must now be measured accurately each time a standard is made. Filling the bag with a constant flowrate for a fixed period of time usually does this. Components can be added to the matrix as it is flowing into the bag. Mixing may be done by gently kneading the bag. Calculations are the same as those for the fixed-volume container. Adsorption problems can be considerable, depending on the

components and the bag material. Bags are also susceptible to small leaks, which can cause serious error, especially in the volume of gas matrix added. It would be reasonable to apply the same time frame of standard preparation and use in fixed-volume containers to bags without other experience (i.e., 1 h).

8.8.2.2 Dynamic Gas Standards

Dynamic methods are basically flow dilution systems providing a continuous flowing calibration gas. In this approach two or more pure gases flow at a constant, known flowrate into the mixing junction. Dynamic standards have two major advantages that make the technique desirable and worth the effort to set them up. The first is that adsorption problems are virtually eliminated in the generation and sampling systems because of the constant flowing system. However, adsorption is not eliminated. It is still present, but very soon the amount adsorbed is in equilibrium with the concentration in the flowing stream. Thus a standard, known concentration is exiting the system. This is extremely important in the preparation of trace standards of reasonably polar and adsorptive materials. The second advantage is that the flowrate of one or more of the components can generally be easily changed, thus providing various concentrations of standards for calibration curves. This becomes important in the initial evaluation of a system for analysis.

For mixtures in the percentage range, the dynamic mixing technique is reasonably straightforward. Flows can be accurately controlled and, with the use of a technique such as a soap-film flowmeter, can be measured reasonably accurately. In general, however, continuous inline flow meters are used, the most common of which are rotameters. It is a very unusual rotameter than can be read and set to within 1% accuracy over even 50% of its scale. Too often the rotameter is read and the value for flowrate is assumed accurate without full appreciation of the reading error involved. In general, the greatest error in dynamic standards is the lack of accuracy in one or more of the flowrates. Again, one should be concerned about pressure and temperature of the gases and that these are the same either actually or by calculation, and also how these two variables affect the means used to measure the volumetric flowrate.

As mentioned earlier, the simple form of dynamic dilution works well in the percent range. However, attempts to produce a 5-ppm (in air) methane standard, by mixing a 1-mL/min methane flow with a 200-L/min airflow, fail simply because of the problems of measuring the high and low flows accurately and conveniently. Generally, a double-dilution technique works here. First, a dynamic standard of 2000 ppm is generated by a flow of 15 mL/min of methane and 7.5 L/min of air. Then 20 mL/min of the 2000-ppm standard is mixed with 8-L/min air to produce 5 ppm. Properly, the airflow to produce the 2000-ppm standard should be 7.485 L/min (7.5−0.015). The total flow of the 2000 ppm is then 7.5 L/min. The same applies, of course, for the second dilution. The equation used to keep these concentrations straight in successive dilutions is as follows:

$$F_1 \times C_1 = F_2 \times C_2 \tag{8.8}$$

where F_1 (mL/min) is the flowrate of concentration C_1 *and* F_2 (mL/min) is the flowrate of concentration C_2. Thus the second dilution given above is

$$20 \text{ mL/min} \times 2000 \text{ ppm} = 8000 \text{ mL} \times 5 \text{ ppm}$$

The accuracy of multiple dilutions fades as an increasing number of dilutions are made because of the added errors of additional flow measurements. In the double dilution given above, four flow measurements are needed, two for each dilution. Fortunately, however, multiple dilutions are used to produce low concentrations where an analysis accuracy of perhaps ±10% would be acceptable.

Low flowrates of gases can be delivered to a larger volume flowrate of a diluent gas by the use of small motor-driven syringes (38). This is one way of accurately delivering low volumetric flowrates. Generally, periods no longer than an hour are used since the syringe must be refilled. Backdiffusion of the diluent gas into the syringe volume at low delivery rates is a problem here. Also, the downstream pressure of the standard thus prepared cannot change since this can cause a pumping action in and out of the syringe volume.

A technique of making known vapor concentrations of reasonably volatile liquids in a diluent gas involves the use of the vapor pressure of the liquid (39). The diluent gas is passed through successive thermostatted bubblers to obtain a mixture determined by the saturation vapor pressure (SVP). Thus for ethanol, if the bubblers were maintained at 20°C (ethanol vapor pressure at 20°C is 43.9 Torr) (6 kPa) and the diluent gas flow were maintained low enough to ensure saturation, a dynamic standard would be generated with the following concentration:

$$C = \frac{\text{SVP}}{\text{total P}} \times 100 = \frac{43.9}{760} \times 100 = 5.78\% \tag{8.9}$$

At this temperature the vapor pressure changes by about 5%/°C, requiring bubbler thermostatting to better than ±0.2°C for a 1% standard accuracy. It is also important to know accurately the total pressure at the final bubbler, since this is also used in the calculation. This was assumed to be 760 Torr (101 kPa), to illustrate the preceding calculation, but must be measured in practice.

If the vapor pressure technique is used, two methods can be used to change the concentration:

1. The vapor pressure can be adjusted by changing the temperature. This can be quite time-consuming in that true thermal equilibrium is required for each concentration. Also, the temperature must be kept lower than any subsequent temperature the developed standard will see to prevent condensation and thus a loss of the standard.
2. The total pressure under which the bubbler system is working can be changed. Since this can be done only for pressures greater than any subsequent pressure to which the standard will be exposed, it can require a sophisticated experimental setup.

In general, for multiple concentrations, one standard is prepared in this fashion and is then diluted by a second diluent gas stream. This requires that both the original bubbler flow and the diluent flow can be accurately measured, whereas with the single concentration provided by the vapor pressure system, the bubbler flow need not be known accurately because it does not enter into the calculations. One only has to be assured that the gas is saturated.

Another approach to vapor standards is to use the diffusion of vapor through a capillary to add small amounts of vapor to a flowing gas stream (40–42). The theory and practice are reasonably well defined. The concentration is determined by knowing the rate of diffusion and using the following equation:

$$C = \frac{R \times K}{F} \tag{8.10}$$

where C = concentration (ppm v/v)
R = diffusion rate (ng/min)
F = diluent gas flowrate (mL/min)
K = 24.45/MW (nL/ng) at 25°C and 760 Torr (101 kPa)

Once again the diluent flowrate must be at the same conditions as the K factor used or vice versa. To obtain an exact ratio of gas volumes, both volumes must be measured at the same temperature and pressure. The K factor simply converts the diffusion rate in weight per unit time to vapor volume per unit time.

Theory predicts the diffusion rate by the following equation (46):

$$r = 2.303 \frac{DMPA}{RTL} \log \frac{P}{P - p} \tag{8.11}$$

where r = diffusion rate (g/s)
D = diffusion coefficient (cm^2/s)
M = molecular weight (g/mol)
P = total air pressure (atm)
A = diffusion cross-sectional area (cm^2)
p = partial pressure of sample at $T°$ (atm)
R = gas constant (mL-atm/mol-K)
T = temperature (K)
L = length of diffusion path (cm)

By incorporating R into the constant, converting both pressures (P and p) into Torr from atmospheres, and converting the rate into ng/min from g/s this equation is obtained:

$$R = 2.216 \times 10^6 \frac{DMPA}{TL} \log \left(\frac{P}{P - p} \right) \tag{8.12}$$

where R = diffusion rate (ng/min)

P = total pressure (Torr)
p = partial pressure of sample (Torr)

All other terms are as given above.

By use of Equation (8.12) with vapor pressures and diffusion coefficients from data in the literature and very accurate measurements of area and length, the diffusion rate generally can be calculated to within ~5%. Thus the only way to build dynamic standards using the diffusion technique is to determine the rate in a given system. One such system is to use a diffusion tube, as shown in Figure 8.11. The bulb of the tube is loaded with liquid to about 80% of its capacity (perhaps 5 mL). The capillary length is variable up to about 7 cm and the capillary diameter perhaps 0.2 cm. This tube is placed in a thermostatted chamber permitting a dilution gas flow across the tube. The diffusion rate is then determined by weight loss over several days using a good analytical balance. Only the gas flowrate need then be measured to generate a primary standard. Diffusion rates can be measured during the life of one filling while the diffusion tube is in use. Different materials can be filled in the same tube, or the tube can be refilled with the same material. Only pure materials can be used, not mixtures. Several tubes, however, can be put in the same gas stream to generate a multiple standard. The concentration of the standard may be varied over a wide range by variation of the dilution gas flowrate. This is preferred to a temperature change of the diffusion tube. Again, the temperature control of the diffusion tube is critical. General practice is to maintain the temperature constant to within $\pm 0.1^{\circ}$C.

Table 8.6 illustrates the types of standards that can be generated by diffusion tubes. The measured and calculated rates give some indication of the errors in attempting to use Equation 8.12 to calculate the diffusion rate. These are primarily the accuracy to which the diffusion coefficient and vapor pressure are known at the operating temperature and the accuracy to which the diameter of the diffusion path and its consistency are known. The measured rate by weight loss is as accurate as the balance used and the ability to hold the temperature constant

TABLE 8.6 Diffusion Tube Data

Chemical	Diffusion Rate[a] (ng/min)		Concentration at 150 mL/min ppm (v/v)
	Measured	Calculated	
Benzene	13,500	14,300	28.2
Toluene	4,550	4,490	8.1
n-Octane	2,160	1,917	3.1
Methanol	15,100	13,700	76.9
Ethanol	6,950	6,840	24.6
Ethyl acetate	15,100	14,200	28.0
Chloroform	45,600	52,200	62.3
Carbon tetrachloride	29,200	33,000	30.9
Acetone	34,500	32,100	97.1

[a] All diffusion tubes were 7 cm × 2 mm i.d., operating at 30°C.

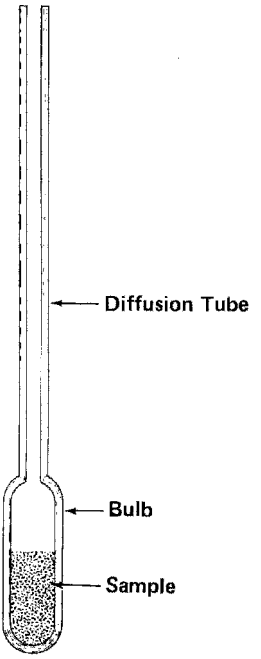

FIGURE 8.11 Cross-sectional diagram of a diffusion tube (courtesy of Analytical Instrument Development).

between weighings. It can easily be within 1% accuracy. The concentrations shown can, of course, be changed by changing the flowrate. Certainly higher concentrations can be developed by using a wider-bore tube, a shorter tube, or higher temperatures. However, the temperature should be held at least 20°C below the boiling point of the material.

An ingenious means of dynamic generation of standards at the ppm level involves permeation through a polymer. In 1966 O'Keefe and Ortman (43) described this technique primarily for air-pollution standards. A condensable gas or vapor is sealed as a liquid in a Teflon tube under its saturation vapor pressure, as shown in Figure 8.12. After an initial equilibrium period, the vapor permeates through the tube wall at a constant rate. This rate is determined by weight loss over a period of time. Temperature must be controlled to within ±0.1°C to maintain 1% accuracy. In use, the tube is thermostatted in a chamber that permits a diluent gas to fully flush the chamber. The concentration is then determined by the same equation used for diffusion tubes:

$$C = \frac{R \times K}{F} \tag{8.13}$$

where C = concentration (ppm, v/v)
R = permeation rate (ng/min)

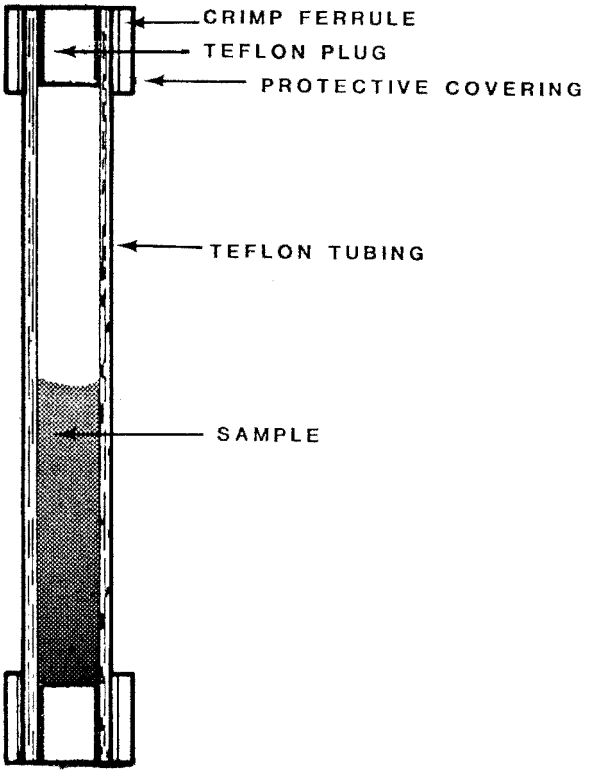

FIGURE 8.12 Cross-sectional diagram of a permeation tube (courtesy of Analytical Instrument Development).

F = diluent gas flowrate (mL/min)
K = 24.45/MW (nL/ng) at 25°C and 760 Torr (101 kPa)

Again, to obtain an accurate ratio of gas volumes, the volumes must be at the same temperature and pressure. Either F is corrected to 25°C and 760 Torr (101 kPa), or the K factor is adjusted to the conditions under which F was measured.

Typical materials available in permeation tubes for operation at 30°C are listed in Table 8.7 along with average rates per centimeter length of tube and the K factor. As the length of the tube increases, the permeation rate increases in reasonable proportion. The data in Table 8.8 are for tubes of 0.25 in. o.d. The wall thicknesses are shown in the table. The final column gives the concentration available from a 5-cm tube using 1 L/min flow. For instance the SO_2 rate is 240 ng/min cm^{-1}. For a 5-cm tube, the rate would be 1220 ng/min. At 1 L/min the concentration is

$$C = \frac{1200 \times 0.382}{1000} = 0.458 \text{ ppm} \tag{8.14}$$

TABLE 8.7 Permeation Rates for Chemicals in Permeation Tubes at 30°C

Chemical	*K*	Thick Wall Thickness (ln.)	Permeation Rate (ng/min cm^{-1})	Concentration at 1 L/min ppm (5-cm Tube)
Sulfur dioxide	0.382	0.062	240	0.46
Nitrogen dioxide	0.532	0.062	1000	2.66
Hydrogen sulfide	0.719	0.062	240	0.86
Chlorine	0.345	0.062	1250	2.15
Ammonia	1.439	0.062	210	1.51
Propane	0.556	0.062	100	0.28
Butane	0.422	0.030	24	0.05
Methyl mercaptan	0.509	0.030	65	0.16
Ethyl chloride	0.379	0.030	56	0.11
Vinyl chloride	0.391	0.030	400	0.78

TABLE 8.8 Permeation Rates for Chemicals in Permeation Tubes at 70°C

Chemical	*K*	Permeation Rate (ng min^{-1} cm^{-1})	Concentration, ppm at 200 mL/min (10-cm Tube)
1,1,1-Trichloroethane	0.183	112	1.03
Trichloroethylene	0.186	1060	9.86
Chloroform	0.205	713	7.31
Carbon tetrachloride	0.159	220	1.75
Acetone	0.422	270	5.70
Methyl ethyl ketone	0.340	100	1.70
Benzene	0.313	260	4.07
Toluene	0.266	120	1.60
o-Xylene	0.231	40	0.46
Cyclohexane	0.291	20	0.29
n-Hexane	0.284	160	2.27
Methanol	0.764	216	8.25
Vinyl acetate	0.284	700	9.94

If the flow is increased to 2.0 L/min, the concentration is cut in half to 0.229 ppm. Longer tube lengths, thinner tube walls, and higher temperatures all increase the permeation. Generally, to prevent the diluent gas from cooling the tube, in practice, a low flow is passed across the tube and is then diluted with a higher flow downstream from the tube. The sum of both flows must be used in the calculation.

Tubes for higher-temperature operation containing some common industrial solvents have been introduced. Some of these are listed in Table 8.8. These

permit low concentration standards to be prepared for some industrial hygiene-type analyses. Some of these tubes at 70°C begin to overlap the type of standards developed with diffusion tubes as shown in Table 8.6. Thus the combination of permeation tubes and diffusion tubes provides means of preparing standards of common solvents from below one to several hundred ppm.

Permeation tubes are not refilled, have a limited life, and cannot be turned off. However, their lifetimes can be prolonged during periods of nonuse by storing them in a refrigerator to reduce the permeation rate. Not many materials are practical for use in permeation tubes. When the technique can be used, however, it is generally preferred as a means of standard preparation.

8.8.2.3 Liquid Standards

Significant space has been devoted to gas standards because of the difficulty in preparing known standards. The fact that such a wide variety of techniques are in use attests to the problem. On the other hand, liquid standards are quite straightforward, and reasonable analytical techniques can ensure reliable standards.

In general, liquid standards are prepared in a solvent matrix, which should be the same as the matrix of the unknown. In many cases the liquid may be an extraction solvent or simply a dilution solvent, depending on the type of analysis (as opposed to being prescribed by a procedure). The solvent should be chosen such that it does not interfere with any of the potential sample components. For trace analysis, it is important that the solvent be checked for impurities and that these impurities not be confused with sample components. Chromatographing the solvent at the maximum sensitivity to be used in the analysis is referred to as "blanking the solvent." It is very important to blank the solvent each time it is used to ensure that it has not been inadvertently contaminated. Also, in trace analysis it is preferred to have a solvent elute from the column following the sample components of interest rather than ahead of the sample.

Standards are prepared by adding known weights of materials to a volumetric flask and then diluting to volume with the solvent or matrix. The approach is best illustrated with an example of the analysis of benzene in toluene at the 0.01% (weight) level. A standard is prepared by weighing 100 mg of benzene into a 10-mL volumetric flask. This is diluted to the mark with benzene-free toluene. This can be used as a master standard. Each milliliter of solution contains 10 mg of benzene. The master standard is then used to prepare several additional standards as follows:

Standard	Master Standard (μL)	Benzene (mg)	Wt/vol (%)	Wt/wt (%)
1	50	0.50	0.0050	0.00577
2	75	0.75	0.0075	0.00866
3	100	1.00	0.0100	0.01155
4	150	1.50	0.0150	0.01732

Each standard is prepared in a 10-mL volumetric flask, and the proper amount of master standard is then diluted to the mark with benzene-free toluene, giving the concentrations shown in the preceding table. The weight/weight percent simply assumes a toluene density of 0.866 g/mL. The calibration curve can now be run by applying the four standards. Peak size can be plotted against absolute weight of benzene in the injected sample or against weight percent, depending on the final form needed for the unknown. Assuming a 1-μL injection, standard 3 would provide a benzene peak for 0.1μg of benzene. This is the convenience of preparing the dilution to volume and calculating weight percent by density. If it is assumed that the density of the solution is the same as the pure toluene, an error of no more than 0.01% relative can be introduced at this level. In this case it is assumed that the density of the unknown is the same as that of the toluene. These assumptions should not be made for solutions in the percent range. At this level the standards should be prepared by weight of each component.

There are several advantages of the double-standard preparation as used above. Significant amounts of toluene are conserved, and the standards are prepared by volume measurements (except for the one weight measurement). Also, other components can be added to the second set of standards at the time of preparation from other master standards. These components can then be varied independently of each other.

Obviously, solid samples can be made up by weight in a solvent as above. This is generally the technique used for such materials as pesticides.

Reliability of liquid standards over a period of time is generally quite good if the standards are kept in sealed containers. They should not be stored for any length of time in volumetric flasks, but small vials are quite convenient. However, a word of caution about the vial caps is in order. Plastic or plastic-coated cardboard liners in vial caps pose serious problems in most cases. Solvents dissolve or leach a number of materials from these caps, generally causing gross interference with the standards. In general, foil-lined caps should be used unless these are known to produce problems.

Tightness of the seal is important to prevent selective evaporation of components or solvent from the vial. Homemade inert cap liners may be inert but seldom adequately seal the vial. Evaporation is generally the major reason why liquid standards become nonstandards. Chemical knowledge of the components should also be considered as far as reactivity and adsorption are concerned in terms of the useful life of standards.

Many small-container designs are available today that form a tight, inert seal and allow sample to be withdrawn by a syringe through a septum. These containers have been known to maintain standards up to a year without change. However, preparation of standards more frequently than once a year is certainly recommended.

8.8.3 Internal Normalization

In the internal normalization technique a sample is injected into the chromatograph and peaks are obtained for all the sample components. Generally, area

measurements are used for all peaks, although peak heights can be used. The basic calculations are shown in Table 8.9 for an assumed sample containing four components: A, B, C, and D. If the peak areas are simply added, one can calculate the area percent. This, however, does not account for the fact that different materials will have different responses in the detector for a given weight. These different responses may be determined either absolutely as concentration per unit area or relative to each other for a given analysis. If one used the area percent as the weight percent, the assumption is that all the components would respond in the detector with exactly the same sensitivity; that is, a given weight of any of the components will give exactly the same area. This might be justified in some cases when one is attempting to check purity of a substance, such as mentioned for standards in Section 8.9. If the major component is 99+%, the error introduced is small.

The data in Table 8.9 are typical of the data for internal normalization. The standard is prepared by adding known weights of the pure components to each other and calculating the weight percent as shown. The standard is then chromatographed and the areas of the four peaks are measured. The area percents are listed to show their relationships to weight percents for this mixture. The weight percents are then divided by areas to give the concentration per unit area. Component A was chosen as a reference and assigned a response factor of 1.000. The other response factors are determined by dividing the concentration per unit area by 0.005138. Generally these response factors should be constant as long as the operating conditions of the detector remain constant. The FID is relatively

TABLE 8.9 Internal Normalization

	Standard					
Component	Taken (g)	Weight (%)	Peak Area	Area (%)	Weight% / Area	Response Factor F
A	0.3786	21.74	4231	22.41	0.005138	1.000
B	0.4692	26.94	5087	26.94	0.005296	1.031
C	0.5291	30.38	5691	30.14	0.005338	1.039
D	0.3648	20.94	3872	20.51	0.005408	1.053
Total	1.7417	100.00	18881	100.00		

	Unknown				
Component	Peak Area	Weight %	Normalized Weight %	Area × F	Weight %
A	3862	19.84	19.66	3862	19.66
B	5841	30.93	30.66	6022	30.66
C	4926	26.29	26.06	5118	26.06
D	4406	23.83	23.62	4640	23.62
Total	19035	100.89	100.00	19642	100.00

insensitive to flow and temperature changes, making it almost ideal for internal normalization. One should be able to reproduce these response factors over long periods of time with this detector. As the response time increases, the detector is less sensitive for that component.

The unknown sample can now be chromatographed and the areas measured. Both approaches mentioned earlier for calculation will be shown. In the first case, each area is multiplied by the weight percent per unit area to obtain the raw weight percents in the unknown. It should be stressed that the sample size injected into the chromatograph was the same in both standard and unknown. When the weight percents are added, however, it is found that the total is greater than 100%. Why? The answer is that the sample sizes were not identical. Sample size is about the biggest error in gas chromatographic analysis next to some of the manual area measurement techniques. The technique of internal normalization corrects for this sample size error. Each weight percent value is divided by the total percent (100.89% in this case) and multiplied by 100 to provide the normalized weight percent. The second approach multiplies each area by the response factor, thus correcting each area for the individual component response factor in the detector. The weight percent is then simply the response-corrected area percent.

Even though this technique can correct the variation in sample size, one should still make the attempt to keep sample size the same. The same sample size then requires a uniform effort on the part of the chromatographic system regarding injection, vaporization, sample loading on the column, and response in the detector. For improved accuracy, component levels in the unknown are bracketed in the standards. Results obtained with the use of this technique on round-robin samples were reported by Emery (44). Emery's paper also provides some excellent data on various methods of peak measurement.

The major disadvantage of this technique is that the entire mixture must be separated and detected in the chromatographic system. All peaks must be standardized by response factors regardless of whether their analysis is needed. Internal normalization also requires that a detector be used that responds somewhat uniformly to all components. This technique cannot be used with electron capture and flame photometric detectors, for instance.

With care, internal normalization can be used where peak size is measured by height instead of area, although this is rare. The response factor is now subject to slight variations in column temperature, injection technique, carrier flow, and the like, all mentioned in the discussion of peak measurement previously. This approach requires that the standard mix for response factors be run as close in time to the unknown as possible. Response factors determined from area measurement are in no way the same as those determined from peak height.

The preceding sample has four components in approximately the same concentration. This is certainly not necessary and in practice is seldom attained. However, a major concern with the use of this technique is that the chromatographic system can handle the absolute amounts injected of all components in a linear fashion. This means that the detector systems must still be responding linearly to the absolute amount of each component, even if one represents 99%

of the sample and is not the component of interest. Certainly smaller sizes can be used, but here again practicality enters in.

One way to avoid the nonlinear problem is to dilute the standard and unknown with a compatible solvent that is fully resolved chromatographically from all the sample components. These dilutions need not be accurately made or be identical for the standard and the unknown. Good practice dictates that they be approximately the same for each. This is merely a technique for injecting a smaller amount of the standard and sample into the chromatograph. Since calculations do not involve sample size, this dilution is not a factor; the solvent and any solvent impurity peaks are not measured and are not to be considered in the calculation.

In theory, the internal normalization technique may appear ideal. But in analysis of real-life samples that may contain many components, some of which may be unresolved chromatographically and of no interest to the analyst, one of the other two techniques offers more advantages and is generally employed. One analysis using this technique and performed hundreds of times each day is the component-by-component analysis of natural gas. A complete analysis is needed since the analysis is used to calculate the heating value of the sample. Thus it is natural to normalize the results.

8.8.4 Internal Standardization

The technique of internal standardization may best be understood by referring to Table 8.9, which outlines the method of internal normalization. It is assumed in this instance that only component C is of interest for analysis and that the unknown contains no component A. If a standard containing known weights of both A and C is prepared and chromatographed, the response factor F can still be determined. This is shown in Table 8.10, assuming the same weights and areas as before. In practice, several standards should be made, with a plot of area as abscissa and weight ratio as ordinate. This plot must be linear for the particular system. Once the linearity is established for a given sample type and system, only one standard mix need be used to define the slope of that plot. Note that the response ratio R is the slope of that line. Therefore, the standard is actually used to determine the ratio R. Note that the response factor F for C in Table 8.9 is the reciprocal of the R ratio in Table 8.10.

The unknown is now ready to be run. Since no component A is present in the unknown, a known weight of this component is added to a known weight of the

TABLE 8.10 Internal Standardization

Component	Weight	Weight Ratio C/A	Area	Area Ratio C/A	$R = \frac{\text{Area Ratio}}{\text{Weight Ratio}}$
A	0.3786		4231		
		1.398		1.345	0.962
C	0.5291		5691		

sample. This mixture is then chromatographed and the area ratio of components C:A is measured. Knowing R, the ratio of the area ratio and weight ratio, and the area ratio in the unknown, one can calculate the weight ratio of the unknown

$$\frac{W_C}{W_A} = \frac{A_C}{A_A} \times \frac{1}{R} \tag{8.15}$$

where W_C and W_A = weights of C and A, respectively, and A_C and A_A are the areas of C and A, respectively; R is the response ratio. Since the weight of A added to the sample is known, the weight of C in the sample can be calculated:

$$W_C = \frac{A_C}{A_A} \times \frac{1}{R} \times W_A \tag{8.16}$$

And since the weight of the sample is known, we obtain

$$\%C = \frac{W_C}{\text{sample weight}} \times 100 \tag{8.17}$$

In practice, a master standard of component A and one of component C are prepared on a weight/volume basis in a solvent. Mixture of known volumes of each of these two standards can provide a variety of weight ratios of the two materials for the initial linearity check. The standard of component A can also be used to add a known amount of A to a known weight of sample.

In the preceding example, area was used to measure peak size since that was the technique used in the example for the internal normalization. Peak height can be used as the size measure just as well as peak area. The same advantages of peak height measurement are present in this method of standardization as in any other. Likewise, the same requirement for frequent standardization is present.

In this instance component A is referred to as the internal standard. All the advantages of the internal normalization technique, such as lack of knowledge regarding the exact sample size and the noncritical aspects of dilution, carry over to this technique. The major disadvantage of internal normalization, namely, the necessity of measuring all the components of the sample, does not carry over into this technique. The cautions under internal normalization regarding system overload apply, but only to the components of interest and the internal standard, not to the entire sample.

Again as with internal normalization, even though the sample size is theoretically not critical, attempts should be made to use the same sample size for both standards and unknowns. This constant load on the chromatographic system gives one the best shot at the high accuracy that the technique of internal standardization is capable of producing.

With attention to the purity of the standards and to the lack of interference of any solvent impurities, the precision of the internal standard method is controlled by the ability to quantify peak size. That certainly qualifies this technique as the most precise method of quantitative analysis by GC, and where

precision is paramount, the internal standard technique should be applied. Its advantages far outweigh the slight increase in effort required for standard and sample preparation.

The preceding discussion of sample storage of external liquid standards certainly applies to the standards prepared for the internal standardization technique. There is one further consideration in this regard, and that is in the proper selection of the internal standard for a given analysis. The first step is to chromatograph a typical sample and identify the component or components to be analyzed. The internal standard is then chosen such that it must

1. Elute from the column adequately separated from all sample components.
2. Elute as near as possible to the desired component(s) and ideally, before the last sample peak so that analysis time is not increased.
3. Be similar in functional group type to the component(s) of interest. If such a compound is not readily available, an appropriate hydrocarbon should be substituted.
4. Be stable under the required analytical conditions and nonreactive with sample components.
5. Be sufficiently nonvolatile to allow for storage of standard solutions for significant periods of time.

Several attempts may be necessary to find the best internal standard for a given analysis, but the effort is worthwhile if highest precision is needed.

8.8.5 Standardization Summary

In all three methods of standardization, standards and samples are chromatographed and the standards are known but the samples are unknown. Peak sizes can then be determined for both. The difference in the three methods is in the second piece of information needed to relate the standard to the sample:

1. In internal normalization this relationship is that in both the standard and the unknown, the analyzed peaks total 100%.
2. In external standardization this relationship is the accurately known amounts of standard and unknown actually injected into the chromatograph.
3. In internal standardization this relationship is the accurately known amount of different material added to an accurately known amount of the standard and unknown.

The errors associated with standardization have been discussed throughout Sections 8.8.1–8.8.4, but should be summarized:

1. Standard purity and known standards must be checked and not assumed. An analytical technique whose principles of measurement are different from those of GC is preferred for the comparison.

2. Linearity of response versus absolute amount injected must be confirmed for each different sample type and each different set of chromatographic operating conditions. This linearity cannot be assumed. Nonlinearity may result from column overload, detector overload, or adsorption problems.
3. Proper attention to good analytical practices is important, especially with regard to proper "blanking" of solvents, syringes, and all sample-handling equipment. The high sensitivity for small amounts of material in most detector systems increases the importance of cleanliness.

8.9 QUANTITATIVE ERROR

8.9.1 General Discussion

Attention has already been given to the errors associated with peak size measurement and standardization. There are many other places in the chromatographic process where errors enter into quantitative analytical GC. Detailed analysis of most of these error sources is not possible, especially in the confines of this chapter, but they should be and are mentioned and briefly discussed. Most of the error sources are generally obvious; it may indeed seem even ridiculous that some have to be mentioned. However, the mere fact that they are obvious tends to slowly place them in the overlooked category. One has to be constantly reminded of these errors until they are routinely or habitually considered in solution of each problem. These general errors can be grouped into two categories: (1) the general area of sampling, involving problems of getting the sample from where and how it is taken and into the gas chromatograph and (2) the gas chromatographic system itself.

An excellent review of the factors affecting gas chromatographic analysis is given by Barwick (45). This review also covers sources of uncertainty with referenced estimates of their magnitudes.

8.9.2 Sampling Techniques

Gas chromatographic hardware and software have evolved to the point, when used properly, where the gas chromatographic system contributes little compared to the sampling error. For example, Barth showed that 92% of the total variance seen in the soil-sampling data came from the sampling and only 8% from the laboratory process (46). Although the soil matrix is probably an extreme example of a difficult and nonhomogeneous system, the data illustrate the importance of sampling to the entire analysis process.

The methods used to obtain samples and physically transport them to the gas chromatograph are really no different for GC than for any analytical technique. However, since GC has the inherent capability to do trace analysis, it becomes even more critical to observe the best analytical sampling techniques. The analyst is seldom directly involved in establishing the sampling protocol. Since many fatal errors can occur at the sampling stage, the analyst should be an integral part of the sampling team. Since the analyst is often considered responsible for the

data from the entire process from sampling to data reduction, it is important that he/she understand the sampling protocol well enough to explain data variability from the sampling as well as the final instrumental analysis. Some major areas of concern are obvious.

The sample taken must be the same sample that one wants to analyze. Since very little sample is required for gas chromatographic analysis, it is very easy to take a small sample that stands a good chance of not being representative of the environment to be analyzed. Small differences in homogeneity, or lack thereof, become quite apparent on two small samples supposedly taken from the same bulk sample.

Problems of adsorption, evaporation, and reaction of samples following the sampling procedure, prior to analysis, must be considered. The discussion regarding storage and handling of gas and liquid standards under external normalization certainly applies even more to the unknown samples. Time between sampling and analysis must be kept to a minimum. In addition, this time element should be checked with standards to ensure that samples do not change with time, or to at least define the extent of the error if no other solution is possible.

Containers for sampling, and indeed all sampling equipment, must be checked to determine the contribution to error. This becomes especially important if the sample must undergo some processing prior to the analysis. This processing may be extraction, preliminary cleanup by column chromatography or even chemical reaction such as esterification. All of these steps must be proved in a given system or known to ensure either quantitative sample handling or reproducibility of the processing. It is not sufficient to assume that if someone obtained 82.3% efficiency in the methyl esterification of adipic acid 3 years ago, then the same efficiency is valid for a procedure that attempts to duplicate that procedure today. Reaction or extraction efficiencies must be reestablished.

8.9.3 Sample Introduction

As mentioned previously, when a known sample size is required, as in the external standardization technique, the measurement of that sample size will generally be the limiting factor in the analysis. However, improper sample injection can introduce into the analysis errors other than those pertaining to sample size. Thus it will be beneficial to examine the various methods of sample injection and both types of error associated with them. A common error source in split-injection systems comes from the discrimination of components in the mixture on the basis of their boiling point differences. The problem can be attributed to in-needle fractional distillation, nonevaporative transport (mist) that bypasses the column inlet, or poor mixing with the mobile phase when low split ratios are used. Errors associated with the inlet system are covered in detail in Chapter 9, “Inlet Systems for Gas Chromatography.”

8.9.3.1 Syringe Injection

The use of a syringe is by far the most common mode of sample introduction into the chromatograph. Today there are a number of excellent syringes on the

market designed for GC. The most common syringe in use today for liquids has 10 μL of total volume. With the current greater use of smaller-diameter capillary columns, coupled with better and more sensitive detectors, sample sizes continue to decrease. Generally, liquid samples of about 1-μL are used. In a sample of this size, a component of interest should be less than 1% of the injected sample. For concentrated samples, this means sample dilution with a compatible solvent. An error can be introduced here if the solvent contains impurities that have the same retention time as any component of interest or if it contains even some of the same material. As with any of solvents in GC, the solvent has to be "blanked" before it is used.

The use of a 10-μL syringe to deliver a 1-μL volume has a certain error associated with the accuracy to which the syringe markings can be read and the plunger set. This uncertainty alone can contribute a 2–5% error in a 1-μL volume. Many users of gas chromatography are acquainted with the problem of injecting a volatile liquid into a hot injection port of a gas chromatograph. The error associated with this phenomenon outweighs the reading error without use of the proper technique. The basic problem is this: With the syringe properly loaded to the 1-μL mark, the amount of liquid contained in the syringe is that of 1 μL in the barrel plus the amount in the needle. When the liquid is injected, the 1 μL enters into the chromatograph, but any of the liquid remaining in the needle after injection and prior to withdrawal also evaporates. This may be the entire volume in the needle, which will be approximately 0.8 μL. The actual volume in the needle can be determined by loading a syringe with a liquid, running the plunger to zero, wiping the droplet off the needle, slowly drawing the plunger back until the liquid–air interface can be seen in the barrel, and then measuring the liquid slug in volume on the syringe. Knowledge of this total holdup on a given syringe can permit one to measure the amount actually injected. If the needle volume is 0.8 μL and the plunger is set at 1.0 μL, the total liquid in the syringe is 1.8 μL. Following the injection the plunger is withdrawn and the amount of liquid remaining in the needle measured. If this now is 0.3 μL, an amount of 1.5 μL was injected, a 1.0 μL by actual injection and 0.5 μL by evaporation from the needle.

There are two problems here. First, four syringe readings are needed (plunger and liquid–air interface, each on initial and final syringe loading), thus giving rise to two reading errors. The second error is worse in that its magnitude cannot be known with certainty. In other words, the amount that is evaporated from the needle may not (and generally is not) representative of the true sample concentration due to selective evaporation of the more volatile components of the sample. A technique used to overcome this selective evaporation is to draw some pure solvent into the syringe (say, 1.5 μL), then about 1 μL of air, about 1 μL of sample, and finally about 1.5 μL of air. The sample slug is then measured in the barrel between the two liquid–air interfaces (two syringe readings). When this material is injected, only pure solvent is left in the needle and the amount that evaporates is not important. All the measured sample volume will be injected.

Another solution to liquid injection is the use of a 1-μL total-volume syringe. This syringe uses the internal volume of the needle for the sample volume. The plunger is a fine wire extending the full length of the needle. The volume readout is actually accomplished on a glass barrel with an indicator inside the barrel much the same as any other syringe. However, the actual liquid held in the syringe is in the needle only. The accuracy of a 1-μL injection is generally within 1% with the use of these syringes, but these syringes are more expensive.

Finally, proper handling technique is very important, especially wiping the outside of the needle and the droplet at the tip of the needle prior to injection. Any residual liquid on the outside of the needle will be caught in the septum puncture and will slowly enter the column. This produces broad tailing, especially of the solvent, making separations difficult as well as introducing an unknown amount of sample. On the other hand, liquid in the needle can be removed by the capillary action of the wiping towel.

All the preceding points regarding liquid injection should be considered even with the use of a standard technique that does not require knowledge of an accurate volume. Selective evaporation cannot be tolerated even with the internal standard method. The size measurement errors obvious from the preceding discussion certainly point to the substantial advantage of the internal standard technique for accurate analysis.

There are reasonably good syringes available today for injection of gas samples. Generally, gas samples are in the range of 1 mL in size. These syringes have a very snug-fitting Teflon plunger, allowing a gastight seal between the plunger and the barrel. A tight, stiff-acting plunger is necessary but not sufficient for a gastight seal. If 99% of the seal is tight, the entire sample can still be lost out of the 1% of the seal that does leak. A tight plunger can give rise to another error. If the needle plugs as a result of particulates in the gas sample, septum coring, or whatever, no sample will enter the syringe and the gas chromatograph. Many “detector malfunctions” have been corrected by syringe needle replacement. A stiff plunger makes a plugged needle difficult to notice. One final comment on gastight syringes. A large number of these have replaceable needles using a standard Luer fitting. This is very convenient for economical needle replacement if the needle becomes burred, bent, broken, or plugged. However, most glass-to-metal Luer fittings will leak gas at 3 atm of pressure. This is a leak source not normally considered but should be the first check placed on a new gastight syringe. One solution is the availability of plastic (Kel-F or equivalent) Luer fittings on syringes and plastic hubs on needles. This combination can greatly reduce leakage problems.

Before concluding our discussion on syringes, we should mention septum problems. The septum is the necessary evil through which a syringe injects samples. Practically every chromatographer has been plagued at one time or another by septum problems. However, it is surprising that these problems are as few as they are considering the function of a septum. A septum must seal, gastight, pressures of up to several atmospheres. It must often seal against helium (the worst-case scenario next to hydrogen). It must do this at high temperatures

($\leq$300°C). And finally it must, under these conditions, maintain a seal during and reseal following repeated piercing in virtually the same place.

The first problem with septa is leakage. Leaks in the system completely destroy the ability to quantify. Septa should be changed on a routine basis (daily) to avoid loss of valuable time and samples. Septum bleed will cause noise and sometimes drift in isothermal operations. In addition to these, in programmed temperature, septum bleed will produce extraneous peaks not dissimilar to those in an impure solvent. Both sensitivity and quantification may suffer. When bleed is a problem, high-temperature, low-bleed septa should be used even though they are considerably more expensive.

If the septum container on the chromatograph is tightened too much (in an effort to ensure leak-free operation), the septum may extrude. This makes it more difficult to pierce with the needle (resulting in bent needles) and invariably results in coring the septum with the core inside the needle. This means a new needle or a new syringe if the needle is permanent. Extruded septa are subject to pieces of the septum breaking off on the chromatographic side, causing increased restriction and possible plugging of carrier flow. It can also cause severe adsorption of sample components on the septum material, making quantification impossible. Tender, loving care of septa with attention to the problems associated with them will provide the chromatographer with peace of mind and much more reliable and expedient quantitative results.

8.9.3.2 Gas-Sampling Valve

With all the problems associated with syringe injection of gas samples, it is not surprising that a more accurate way of injecting gas samples is generally used. This system makes use of a gas-sampling valve. There are a number of these valves on the market using either rotary or push–pull actuation. Interchangeable volumes are standard. A schematic for a rotary valve is shown in Figure 8.13. In the load position, the volume of the valve is connected to the "in" and "out" load ports. In use, the sample is pulled through the valve by a pump, squeeze bulb, or even a syringe used in the suction mode. If the gas is under pressure, it is allowed to flow through the valve. Sufficient volume of gas is needed to ensure that the "loop" or valve volume contains the sample to be analyzed. For a 1-mL sample loop volume, generally 10 mL of gas is a sufficient flush. The valve is then rotated to the inject position. This action places whatever is in the valve loop into the carrier-gas flow, where it is carried directly to the column for separation. The biggest error, namely, that of volume, is now fixed. If standards are run with the same fixed volume as the sample, the actual volume need not be known with a high degree of accuracy since it is the same for both standard and unknown and will cancel out of the calculation. However, two other parameters must also be held constant in the use of the gas-sampling valve to ensure that the same amount of sample is injected of standard and unknown: temperature and pressure. Either a 3°C difference or a 7-Torr (0.9-kPa) difference will cause a 1% change in the amount of gas sample. The practical solution of these variables is simply to run standards and unknowns as close in time as possible such that these parameters do not vary significantly. If the samples are hot, such as stack

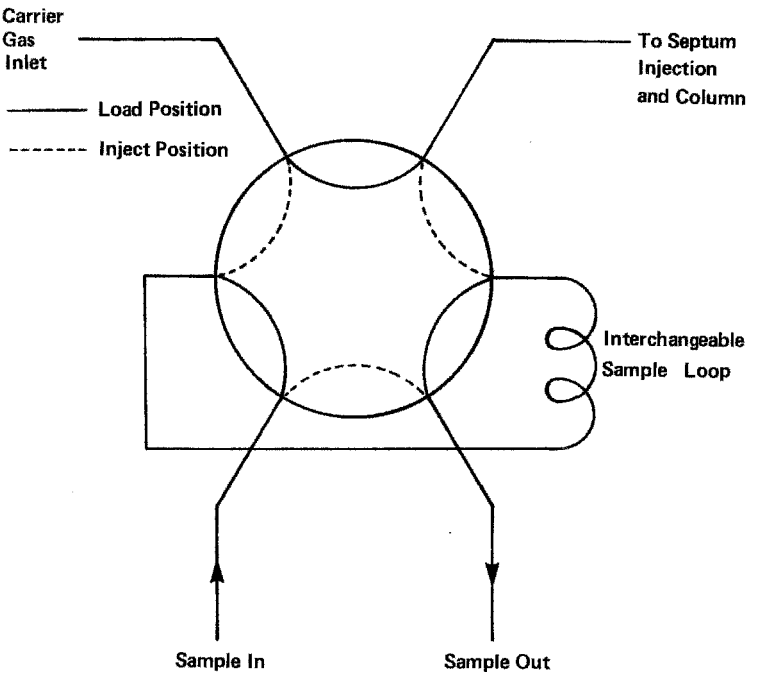

FIGURE 8.13 Flow schematic of a typical rotary gas-sampling valve.

gases, it may be necessary to maintain all sample lines and the gas sampling valve at an elevated temperature. Obviously, standards must be sampled at the same elevated temperature. If the sample is under reduced pressure, the pressure is then usually measured by use of a manometer to provide proper correction, or, preferably, to permit the standard to be handled at the same pressure.

It may be tempting to increase the loop volume to increase the amount of sample for trace analysis. Before this is done, the system should be examined. If $\frac{1}{8}$-in.-diameter columns are used, a reasonable flowrate is 30 mL/min at atmospheric pressure. But if the pressure at the head of the column is at 3 atm (300 kPa) (which is not unreasonable), the volumetric flow at the head and through the sample loop is only 10 mL/min, or 6 s/mL. If the loop volume is increased to 5 mL, it will take 30 s to sweep the sample onto the column. Thus no peak can be any narrower than 30 s. A large-volume loop can completely destroy the separating efficiency of the chromatographic process. Again, as with any analytical problem, a common sense, logical examination of the whole picture will pinpoint problem areas.

8.9.4 Gas Chromatographic System Errors

Most of the problems associated with the processing of the sample through the column and then its detection are basically covered in specific chapters of this

book. However, some areas deserve special mention as they relate to quantitative analysis.

The major concern is that the character of the sample is not changed in the injection port, the column, or the detector before it is actually detected. Thermal decomposition, catalyzed or thermal reaction, and adsorption of part or the entire sample will contribute to error in the analysis. Problems such as these may be determined by using the chromatograph itself first to detect possible problems by unexpected results and then confirmation of the problem by variation of the actual operating parameters of the chromatograph.

Adsorption problems are generally indicated by failure of the calibration curve to pass through the origin, and in some cases by nonlinearity of the curve. A change of the column may be the answer. Perhaps increased temperature will reduce the problem to a workable level. Even though it is not desirable, some adsorption can be tolerated and still give quantitative results, but frequent recalibration is critical.

Sometimes thermal decomposition and reaction can be shown by variation of injection port temperature, and possibly column temperature. The only real solution is to operate at as low a temperature as possible and perhaps use on-column injection. In all cases the precision and accuracy of the quantitative analysis will be affected until a solution is found or a decision is made to "live with it."

Detector errors are basically concerned with the time constant of the detector and its linearity. The time constant certainly can affect the peak height on narrow, sharp peaks, and this may or may not show up as nonlinearity. Assuming a good detector system, the basic linearity concern is with overload. This points to the necessity of initially establishing a calibration curve and assuring its linearity over the entire range of the samples. Extrapolation is dangerous.

8.10 VALIDATION OF GAS CHROMATOGRAPHIC SYSTEMS

Once the gas chromatographic method has been developed, it is often necessary next to prove that the method measures what is needed and intended. A check of expectations for the quality of the data and an action plan to address the results that do not conform is also required. The process by which a method is tested by the developer or user for reliability, accuracy, and preciseness of its intended purpose is called validation (47) (see Chapters 7, 17). The reviewer must look at factors such as reproducibility, accuracy, bias, ruggedness, limit of detection, sensitivity, selectivity, spike recovery, linearity, dynamic range, limit of quantitation, and stability or drift. Although most chromatographers would agree that these are important parameters, there is no universal agreement on how to perform these measurements. Depending on whether the analyte is from an environmental or pharmaceutical project, the approaches can be quite diverse. The important point, however, is to perform the validation so that the analyst and validator agree on what is needed so that the expectations are clear. Otherwise

many resources could be applied to the validation with little potential for satisfaction. The *Guidance for Industry* (48) document provides an excellent resource for method validation, especially for chromatographic techniques.

In summary, GC is an excellent analytical tool for quantitative analysis. However, common sense must be used in handling problems, and the entire system should be understood. The best technique should be used to standardize and for sample handling. The "weakest link" concept is no more pronounced that it is in quantitative GC.

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CHAPTER NINE

Inlet Systems for Gas Chromatography

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9.1 INTRODUCTION AND OVERVIEWS

> A detailed investigation of the phenomena related to the solvent effects runs the risk of creating a picture so complex that it will frighten most chromatographers. . .
>
> —Grob (1)

> I believe that injection has never been optimized with sufficient professionalism. . .
>
> —Grob (2)

> It is my impression that GC injection techniques are still far from being optimized to the point which could be reached. . .
>
> —Grob (3)

In gas chromatography, sample injection, while perhaps the most important (columns and detectors are not of much use if there is no sample introduction) aspect of the instrumentation, remains somewhat a mystery to most chromatographers. It is easily surmised that liquid phase samples, when transferred using a syringe into a hot inlet, vaporize, mix with carrier gas and transfer to a column; however the many physical processes that accompany these transfers are not well understood, even though these have direct and important effects on quantitative analysis and reproducibility. In this chapter the major sample introduction techniques for gas chromatography are described, with details on instrumentation, operation, consumables and method development. General guidelines for choosing the correct inlet are provided.

Throughout this chapter, the following definitions are used. *Injection* refers to the entire process of transferring a liquid sample from a syringe through the inlet and into a column. An injector is the device (autoinjector or human) that performs the physical task of transferring the sample from a container into the GC. An inlet is the device [packed direct, split, splitless, cool on-column, programmed-temperature vaporization (PTV)] on the gas chromatograph that accepts the sample and transfers it to the column. The focus of this chapter

will be on injection techniques and inlets that employ syringes to perform the injection, as these are, by far, the most commonly used devices.

There are several excellent textbooks dedicated to capillary gas chromatography inlets and injection techniques. A perusal of these references highlights both the blessings and problems involved with inlets and sampling for gas chromatography. K. Grob provides the most detailed examination of split and splitless inlets in the several editions of his text, *Split and Splitless Injection in Capillary Gas Chromatography*; the most recent published in 2002 (4). K. Grob also provides an excellent text relating to on-column and PTV injection (5) Janssen provides a straightforward primer on capillary gas chromatography inlets, with an emphasis on large volume injection with PTV inlets (6). Although most capillary gas chromatography inlets are fundamentally similar, there are subtle differences in their operation. The analyst should certainly read the inlet manufacturer's literature, instructions, and recommendations carefully.

9.1.1 Fundamental Problems with Capillary Injection

When gas chromatography with packed columns was developed in the 1950s, injection was relatively simple, and this simplicity continues today. Syringe needles easily fit within the bore of the $\frac{1}{4}$- and $\frac{1}{8}$-in.-o.d. tubing commonly used with packed columns. Therefore, when using a syringe to inject into a packed column, the entire amount of sample that leaves the syringe enters the column, with no need for complex valves or pneumatics.

When capillary columns were invented, the first fundamental problem occurred—*the syringe needle no longer fit inside the column!* This basic difficulty has led to all of the capillary inlets described in this chapter. A special interface, between the syringe and the capillary column, where sample evaporation, mixing with the carrier gas, and transfer to the column, is therefore needed. The second fundamental problem is the mass problem. Packed columns generally contain grams of stationary-phase material, meaning that there is little chance of overloading the column with a typical $1-\mu L$ liquid injection, which weighs about 1 mg. However, a capillary column contains only a few milligrams of stationary phase, meaning that a 1-mg injected sample has a mass very similar to the stationary-phase mass, leading to overloading. This led to the development of inlet splitting and the split inlet, which significantly complicated the pneumatics in capillary GC.

The development of splitting led to the third fundamental problem: detection limits. If it is assumed that 1 μL of a liquid sample weighs about 1 mg, then 1 ppm of that sample weighs about 1 ng. With many common detectors having limits of quantitation in the high-picogram range, it is easily seen that a common 1-μL injection provides a best-case (with no splitting) limit of quantitation of about 1 ppm or a little less, which is far too high for trace analysis applications. With splitting, this limit becomes even higher. Therefore, many gas chromatographic methods are complicated by the need for extensive sample preparation, or the need to interface the gas chromatograph with another instrument or robotic sampler that prepares and concentrates samples prior to injection.

Additional fundamental problems arise from the physical manipulations of the sample that occur during gas chromatographic injection. These can lead to the loss of some sample components, but not of others, termed "discrimination". First, discrimination results from heating of the syringe needle as it enters the inlet, typically causing high-molecular-weight compounds to be retained in the needle, rather than injected. Similar discrimination may also occur in the inlet. Further, lower-molecular-weight compounds may be carried away by the pneumatics if too much sample is injected (inlet liner overload), or if flows are not set correctly. Analysts should be aware that 1 μL of liquid sample will generate 200–1000 μL of vapor, depending on the inlet conditions and the solvent. If the vapor volume is larger than the inlet liner volume, then vapor overload causes sample loss. Finally, labile compounds may react with or adsorb on heated inlet components.

9.1.2 Overview of Capillary Inlets

Four inlets are in common use in capillary gas chromatography today: split, splitless, on-column, and programmed-temperature vaporization. These four inlets lead to myriad injection techniques and methods; the most common of these are discussed in detail in this chapter. Split and splitless injection are both performed using the same inlet, which is often termed a *split/splitless inlet*. Care must be taken when describing experiments, as the split/splitless inlet is capable of performing only one technique at a time; it can perform either split or splitless injection in a single analysis, not both. Each inlet and technique requires a different approach to method development and optimization and is applicable to different sample types. All the available inlets and injection techniques have advantages and disadvantages that must be studied and tested systematically.

9.1.2.1 Split

The split inlet is designed to solve the first two fundamental problems: that the syringe needs does not fit into a capillary column and that a full microliter of a liquid sample may overload the stationary phase. A split inlet allows the introduction of a user-selectable fraction of the injected sample into the capillary column by adjusting the relative flows of carrier gas into the column and to waste through a purge valve. Split inlets are heated, with a high thermal mass, to ensure that the entire injected sample evaporates quickly and mixes homogeneously with the carrier gas. Injection using a split inlet is the classical sample introduction technique in capillary GC and is by far the simplest technique for relatively concentrated (ppm and higher) samples; however, the split inlet suffers tremendously from the mass problem. Because of high carrier-gas flowrates through the inlet, split provides the most rapid injection of all the techniques, leading to the narrowest initial bandwidth on the column, and is the technique of choice for small-diameter columns and rapid separations, which require rapid injection.

9.1.2.2 Splitless

The splitless inlet, which employs the same instrumentation as the split inlet, provides a means for improving sensitivity by transferring nearly the entire injected

sample into the capillary column, rather than venting most of it through the purge vent. Basically, spitiless injection is performed using the same instrumentation as split, except that the purge valve is closed at the moment of injection and remains closed for a period of time (typically 30–60 min) following the injection. During this period, the sample vapor has no place to go but into the capillary column. When the purge valve is opened, any sample vapor remaining in the inlet is rapidly swept out of the purge valve. Typically, about 95% of the injected sample reaches the capillary column, with sample overload and peak broadening avoided through a series of complex phenomena, related to flow, thermal, and solvent effects. Injection using the splitless inlet is the most common means for improving detection limits and is currently the most commonly used technique for trace (low ppm and ppb analyte concentrations) analysis, although complex sample preparation is often still required, and method development can be difficult.

9.1.2.3 On-Column

An on-column inlet is designed to allow the placement of the entire sample directly into a capillary column, without a separate vaporization chamber. This typically requires a special syringe and can now be preformed routinely. In a manner similar to that for splitless injection, the entire sample reaches the column and the analytes are separated from the solvent through thermal and solvent effects. On-column injection is a non-vaporizing technique, as the sample reaches the column as a liquid, which is later vaporized by temperature programming the column and/or the inlet. Because of the need for special syringes and care in sample preparation, the on-column inlet may not be practical for all situations, but it is the technique of choice for the best quantitative analysis. Dirty samples may be problematic, as nearly all sample material reaches the column. A retention gap can partially mitigate this problem. With the addition of a solvent vapor exit, on-column injection can be modified to allow the introduction of up to hundreds of microliters in a single injection.

9.1.2.4 Programmed-Temperature Vaporization

A programmed-temperature vaporization (PTV) inlet is a hybrid of the techniques described above. It is a split/splitless inlet that has been modified to allow cold injection and rapid temperature programming. Similar to on-column injection, the injection occurs while the inlet is cold. In contrast, the injection is performed into a chamber, similar to the split and splitless techniques. This chamber is then rapidly heated to desorb the sample into the capillary column. This inlet also allows for the injection of up to hundreds of microliters of sample. There are numerous modes in which a PTV inlet can be operated, making it perhaps the most versatile of all available inlets.

9.1.3 Overview of Method Development Issues

While each inlet described above has its own individual method development issues and good operational practices, each presents a common problem in the

initial choice of technique, based on the sample type. The initial choice of inlet is not trivial; however, it is often driven by availability on the instrumentation in the laboratory. Split and splitless inlets are far more common than on-column or PTV, so they are nearly always tried first. Guidelines for choosing an inlet on the basis of a few common sample parameters are listed in Table 9.1. As is easily seen, in most cases, on-column is the inlet of choice, however, it is not commonly used. It is interesting that there is no amenable inlet for ultra-trace-level samples, which are of great research interest today. This lends credence to the quotations at the beginning of this chapter.

9.1.4 General Considerations for Proper Injection

Before discussing the specific inlets and injection techniques, it is necessary to briefly review some proper operating principles for gas chromatographs that may affect the injection and sampling process. In fact, nearly all aspects of gas chromatograph operation may affect the injection process. For example, the choice of

TABLE 9.1 Chart for Choosing an Inlet Based on Sample Type

Sample Concentration	Sample Stability	Analyte Boiling Point	Solvent Polarity	Technique of Choice
High (ppm)	Unstable	High	Polar	On-column or PTV
			Nonpolar	On-column or PTV
		Low	Polar	On-column or PTV
			Nonpolar	On-column or PTV
	Stable	High	Polar	Split or splitless
			Nonpolar	Split or splitless
		Low	Polar	Split or splitless
			Nonpolar	Split or splitless
Low (ppb)	Unstable	High	Polar	On-column or PTV
			Nonpolar	On-column or PTV
		Low	Polar	On-column or PTV
			Nonpolar	On-column or PTV
	Stable	High	Polar	Splitless, PTV or on-column
			Nonpolar	Splitless, PTV or on-column
		Low	Polar	Splitless, PTV or on-column
			Nonpolar	Splitless, PTV or on-column
Very low (subppb)	Unstable	High	Polar	?
			Nonpolar	?
		Low	Polar	?
			Nonpolar	?
	Stable	High	Polar	(large volume) PTV or on-column
			Nonpolar	(large volume) PTV or on-column
		Low	Polar	(large volume) PTV or on-column
			Nonpolar	(large volume) PTV or on-column

column dimensions greatly affects the flows and pneumatic settings, influencing inlet performance. The choice of detector, especially mass spectrometers, which operate under vacuum, versus atmospheric pressure detectors, such as FID, will also affect the flows at the inlet. These are described elsewhere in this text and chapter, so they are not described in detail here.

First and foremost, "capillary GC is clean GC" (7). Many of the problems and compromises used in capillary inlets are the result of needing to ensure that "clean" samples reach the capillary column. As with columns and detectors, it is important to ensure that carrier-gas supplies used in capillary inlets are of high purity, are connected to the gas chromatograph using two-stage regulators and have proper scrubbers. Recommendations on these from the vendor of the gas chromatograph and/or the column should be followed. Most new capillary gas chromatography instruments employ electronic control of all flows and pneumatics, while most gas chromatographs built before 1995 employ manual pneumatics. Microprocessor-controlled pneumatics allow much greater flexibility than do manual pneumatics, as pressures and flows can be changed during the analytical run; with manual systems, flows are limited to whatever is set at the run start. For example, with an electronically controlled inlet, the inlet pressure can be elevated at the first part of the analysis to ensure a rapid injection, and then can be reduced to provide the optimum column flow during the separation. Especially in PTV inlets, pressure, flow, and valve changes can be varied in a complex fashion, if needed, during method optimization. Additional considerations common to nearly all inlets include syringes, consumables, and the various ferrules, connectors, and fittings.

9.1.4.1 Syringes

In most cases, liquid and gaseous samples are introduced into the gas chromatographic inlet using a microsyringe. For liquid samples, these typically have volumes of 0.1–10 μL and for gaseous samples, volumes are typically 10–1000 μL. Syringes may include Teflon-tipped plungers to maintain inertness and to maintain a gastight seal. For each inlet and injection technique, there is a recommended syringe, so the analyst should consider the inlet manufacturer's recommendations. For on-column injection on capillary columns, syringes with tapered needles that can fit inside the small column opening are used. Syringes with reinforced plungers and needles have become available, reducing problems with breakdowns. Syringes should be checked regularly for leaks and mechanical failure and should be replaced if either are suspected.

Since the early 1960s, there has been much discussion in the literature of syringe technique during the injection process. Most of this related to facilitating rapid and quantitative sample transfer into the inlet, despite slow, nonreproducible manual operation. These techniques, including cold needle, hot needle, solvent flush, and inclusion of air along with the sample, are not discussed in detail here, as for most hot injection methods (split and splitless), a fast autoinjector, readily available for all gas chromatographs and used as the standard in most industries, will provide excellent quantitative injections. For more information on manual injection techniques the reader is referred to Reference 3.

9.1.4.2 Consumables

Proper care and maintenance of all gas chromatographic inlets requires an extensive supply of tools and consumables. These may include septa, glass sleeves, o-rings, graphite or graphitized vespel ferrules, glass wool, packing materials, fittings, and wrenches. For ease of maintenance, a kit containing all of these items, as listed by the inlet or gas chromatograph manufacturer, should be maintained with each system.

9.1.4.3 Ferrules, Connectors, and Fittings

Making the proper connections, especially in the installation of a capillary column, is critical to the good practice of gas chromatography and to avoiding problems. The two most important considerations are that the capillary column be properly cut and that the column end be inserted the correct distance into the inlet. Proper column-cutting techniques are reviewed in the literature provided by the column manufacturers, and the inlet manufacturer provides the proper installation procedure. Note that these procedures may vary from manufacturer to manufacturer, so they should be studied carefully prior to installing a column.

When making connections, most graphite and graphitized vespel ferrules need to be tightened only fingertight plus at most $\frac{1}{4}$ turn. Overtightening ferrules is the most common cause of leakage in inlet connections. Also, when using connectors, there should be no debris or other contaminants present, as these also cause leaks and poor peak shapes. An electronic leak detector should be used to check for leaks; soap solutions should never be used, as these can diffuse into the tubing causing contamination.

9.2 PACKED-COLUMN INLET

Packed-column instruments offer the simplest sampling devices, as the fundamental problem of the syringe needle diameter is not an issue; all common gas chromatographic syringes will fit inside a packed column. This inlet only requires that the samples be introduced without generating leaks, that it be heated to vaporize the sample and that the carrier gas be able to flow into the column.

9.2.1 Description and Instrumentation

A schematic diagram of a packed-column inlet is shown in Figure 9.1. This may be configured as a direct inlet, in which sample is injected into a short glass sleeve that is butt-connected to the column, or as an on-column inlet, in which the sample is injected directly into the empty column end. The on-column configuration is shown in the figure. The entire inlet is heated to a temperature high enough to completely and rapidly vaporize the injected sample. Carrier gas, typically mass-flowrate-controlled, flows into the inlet and around the outside of the column, to come to thermal equilibrium with the inlet. The syringe needle pierces a septum, to deliver the sample without leaking. The injected sample vaporizes in the head

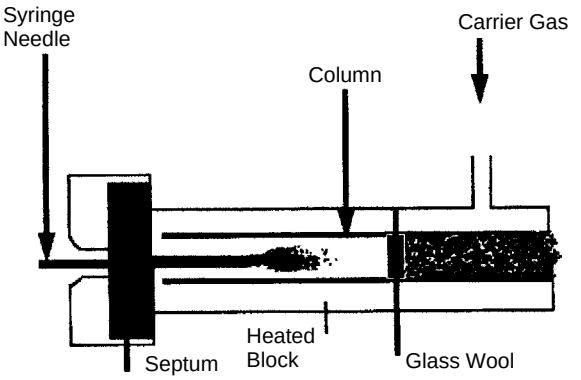


FIGURE 9.1 Schematic of a packed-column inlet. Carrier gas flows into the inlet, around the column, and into the column head. The syringe pierces through a polymeric septum. The entire inlet is heated. (Figure courtesy of Prof. Harold M. McNair).

of the column, mixes with the carrier gas, and is transferred to the stationary phase. Glass wool or a frit is used to hold the stationary phase material in place.

9.2.2 Method Development Considerations

For effective sample evaporation, the inlet temperature is usually set at least 50°C above the normal boiling point of the sample solvent, or equal to the elution temperature of the latest-eluting sample component. The temperature must be balanced against too high a temperature causing syringe needle discrimination (described in Section 9.1.1), sample decomposition or excessive solvent vapor volume. The most inert configuration involves the insertion of a glass packed column all the way into the inlet, with the injection occurring directly in the column. The inlet flow rate is typically set to the optimum flowrate for the capillary column.

9.2.3 Advantages

The main advantage of the packed column inlet is that the entire sample that exits the syringe enters the column, making packed column injection highly reproducible. The pneumatics are also very simple and inexpensive. Method development is also very straightforward with only the inlet temperature as an easily adjustable variable. Further, packed columns and inlets typically operate at lower temperatures than capillary inlets, allowing the use of less expensive septa.

9.2.4 Disadvantages

The main disadvantages of packed-column inlets arise from contamination from nonvolatile sample components or septum pieces. Since the column is usually

placed directly into the inlet, these materials may contaminate the column head. Since packed-column inlets are hot, they may degrade thermally labile compounds. While it is possible to modify a packed-column inlet to accommodate a 0.53-mm-i.d. capillary column, using smaller diameter capillary columns is not practical.

9.3 SPLIT INLET

The first technique for placing an appropriately sized sample into a capillary column is called "splitting" or "split injection." On most gas chromatographs, a single inlet, capable of performing both split and splitless injections, is standard equipment. In a split injection, a liquid (0.1–2-μL) or gas (50–1000-μL) sample is rapidly delivered into a heated glass chamber, typically 2–4 mm inside diameter and a few centimeters long. The exact dimensions of the glass liner depend on the instrument manufacturer. Ideally, the entire sample is vaporized rapidly and homogeneously mixed with the carrier gas. The chamber has two outlets: the capillary column, which has a small diameter; and the purge vent exit, which has a larger diameter. The vent includes a needle valve that controls its flow and regulates the ratio of vent flow to column flow, the split ratio. The pressure drop between the column head, at the inlet and the outlet at the detector, determines the column flowrate.

9.3.1 Overview of the Instrumentation

A schematic diagram of a typical split inlet is shown in Figure 9.2. While each brand of instrument's configuration is slightly different, for most practical purposes, they are all variations on these common themes. First, carrier-gas flows in to the top of the inlet, just below the septum. Here, the carrier-gas flow splits between a septum purge vent and the glass liner. The septum purge is a slow flow, typically a few milliliters per minute, that passes underneath the septum

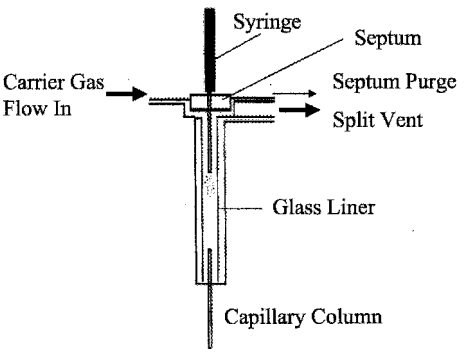


FIGURE 9.2 Schematic of a Split Inlet.

and is vented, to prevent any materials desorbing from the septum from entering the inlet and the capillary column. The other flow path goes into the glass liner, where the syringe needle deposits the sample. In a split injection, there is usually a large (typically 50–100 mL/min) flow of carrier gas through the glass liner. Ideally, the injected sample will be vaporized and mixed with the carrier gas. At the end of the inlet liner, there are two possible exits: the capillary column and the purge vent. A capillary column typically has a relatively low volumetric flowrate (about 1 mL/min), which is determined by the column head pressure setting and the column dimensions, and the purge vent has a higher flow (typically 50–100 mL/min), which is controlled by a needle valve.

The ratio of the volumetric flowrate out of the purge vent to the volumetric flowrate in the capillary column is termed the *split ratio* and provides an estimate of and control over the actual volume of sample entering the column. Care should be taken when using the split ratio to estimate actual injected sample volume, or when using it in comparisons between methods on different instruments. There are subtle differences between instruments and measurement techniques that may affect the measured flows. For example, the column volumetric flowrate measured by injecting a nonretained substance is the average column flowrate, not the flowrate at the inlet, while a flowmeter connected to the split purge vent measures the volumetric flowrate at the vent, not in the inlet. With newer, electronically controlled systems, the flows are measured directly at the inlet, or are calculated from the entered inlet conditions and column dimensions.

9.3.2 Basic Operation

To set up an inlet for split injection, several tools and consumable supplies are needed. These are often provided with the installation kit for a new instrument, or are available from an aftermarket supplier. It should be noted that each instrument vendor's inlet is different, so it should not be assumed that connectors and supplies are exchangeable between instruments. While proper installation of the capillary column and setup of the gas supplies are critical, these are addressed in the instrument manufacturers' documentation, so they are not addressed here. The necessary supplies include appropriate (English or metric) wrenches for making connections, glass liners, septa, O-rings and seals, ferrules and fittings for connecting and installing columns. Generally, maintaining capillary inlets is straightforward, provided maintenance is performed on a regular schedule, according to the manufacturer's instructions.

9.3.3 Septa

The septum presents one of the most convenient but problematical of the basic components of a capillary inlet. It is located at the top or the front of the inlet and is the most commonly replaced part. Its role is to provide a means for conveniently introducing the sample without causing the system to leak or requiring special valves. Septa generally require replacement every 30–50 injections,

depending on the injection technique and type of syringe. Wider-bore and blunt needles require that the septum be replaced more often. For capillary inlets, septa manufactured from high-temperature stable polymers should be used; temperature tolerance should be checked in the septum manufacturer's literature prior to use. Improper septa will decompose under the high-temperature conditions of capillary inlets, causing baseline disruption and ghost peaks in a chromatogram.

Chromatograms showing bleed profiles of several septa are shown in Figure 9.3 (8). Septum bleed is typically observed when temperature programming and usually occurs through the middle of the temperature range. Further, septum bleed is an indication of aging of a high-temperature septum and is an indication that the septum should be replaced. Finally, when installing a septum, the septum nut generally does not need to be tightened beyond fingertight. When running quantitative methods, the septum should be changed often, as particles from worn septa can fall into the inlet and potentially react with or adsorb analytes. Whenever a septum is changed, it is advisable to also inspect the glass liner for septum particles. If these are present, then the glass liner should also be replaced.

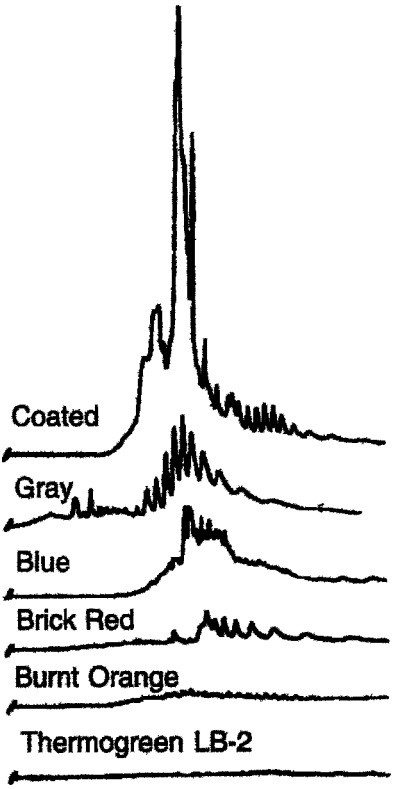
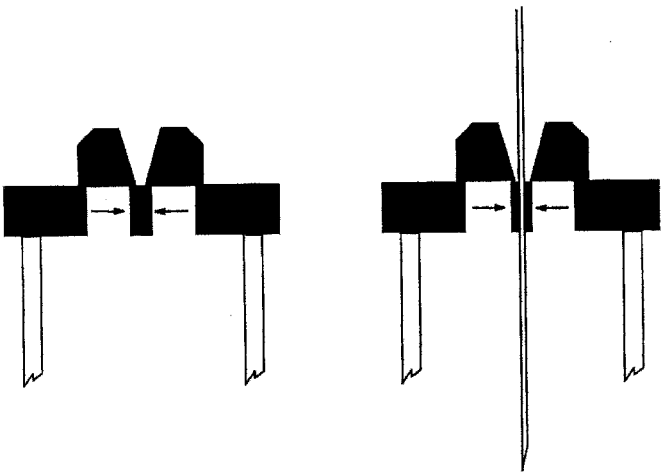


FIGURE 9.3 Chromatograms showing septum bleed from various septa. Each type of septum is designated by a different color. [Adapted from the Supelco Catalog (Supelco, Bellfonte, PA), 2000].

There are potentially effective alternatives to classical septa; the main advantage is that they do not require replacement for up to thousands of injections. One option is a duckbill valve through which the syringe needle passes (see Figure 9.4). This can be fitted onto a standard septum nut, replacing the septum without further modification of the inlet. The main disadvantage of this septum replacement is that it can leak if the syringe is not precisely aligned, as an off-center needle does not fit well into the duckbill. The duckbill valve forms the basis for the commercially available Merlin Microseal device, which is designed to replace the septum and septum nut on most gas chromatographs. A second, more complex, alternative consists of a jade valve, in which the syringe needle displaces a jade ball and passes through a needle guide to maintain an effective seal. A schematic of a jade valve is also shown in Figure 9.4.

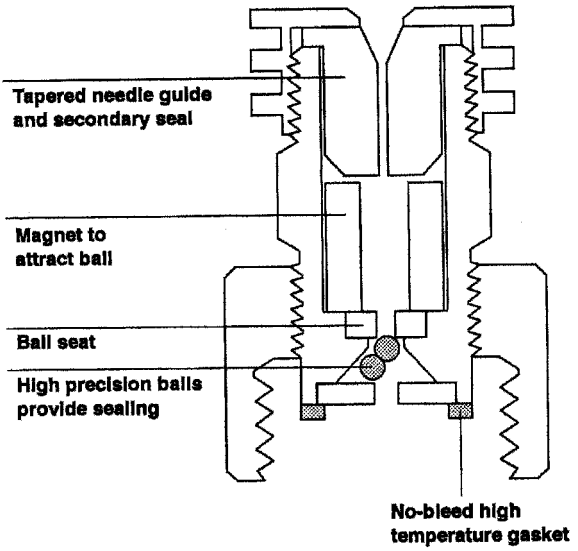
9.3.4 Glass Liners

The glass liner provides a space for the injected sample to vaporize, mix with the carrier gas, and transfer to the column. Thus, proper selection and maintenance of the glass liner is critical to successful capillary gas chromatographic injections. Generally, the glass liner should be inert, have sufficient volume to accommodate the vaporized sample, and should have an obstructed flow path to aid vaporization and to prevent sample liquid from directly entering the capillary column. Each model of gas chromatograph (and sometimes different gas chromatographs from



(*a*)

FIGURE 9.4 Alternatives to polymeric septa: (a) a "duckbill valve" uses an elastomeric flap that is held closed by carrier-gas head pressure, but that allows easy, nondestructive penetration by a syringe needle; (b) a "jade valve" uses a magnet and two steel balls to seal pressure in the inlet. The balls are displaced during syringe introduction, and the needle guide provides seals with the syringe needle. The needle guide must be carefully matched to the syringe needle to prevent leakage.



(*b*)

FIGURE 9.4 (*Continued*)

the same manufacturer) requires a glass liner of different dimensions (length, outside diameter), so care should be taken in selecting the proper one. When installing a glass liner, it is important to use the correct O-ring seals and other fittings and not to overtighten, as the glass can be compressed and cracked.

Figure 9.5 shows a few of the myriad geometries for the inside of the glass liner for split injection. Each is designed to promote vaporization of the sample and mixing with the carrier gas. Most of the heat used in vaporizing the sample is transferred from the glass walls, not from the gas inside the liner, so a high surface area is generally used to aid in heat transfer. The basic glass liner for split injection is shown at the top of Figure 9.5. The inverted glass cup toward the column end provides a high surface area and an obstructed flow path. This may also be packed with glass wool or with stationary-phase material, to provide additional surface area and to facilitate capture of nonvolatile material in the inlet, as opposed to the column. The final example is a straight glass tube, packed with glass wool, which is the easiest to maintain, as it can be readily cleaned.

Glass liners are often chosen by experimentation. In general, simpler systems are preferred; begin with one of the basic designs, such as the cup design at the top of the figure and then working toward a more complex configuration as needed. There is little systematic analysis of this choice in the chemical literature.

For trace quantitative analysis, the glass liner must be highly inert and free of debris or contaminants. It is worthwhile to check the glass liner for contaminants anytime the septum is replaced or when column maintenance is performed. Inertness of glass liners can be improved by purchasing deactivated glass liners that have been pretreated with silylating reagents to eliminate the surface

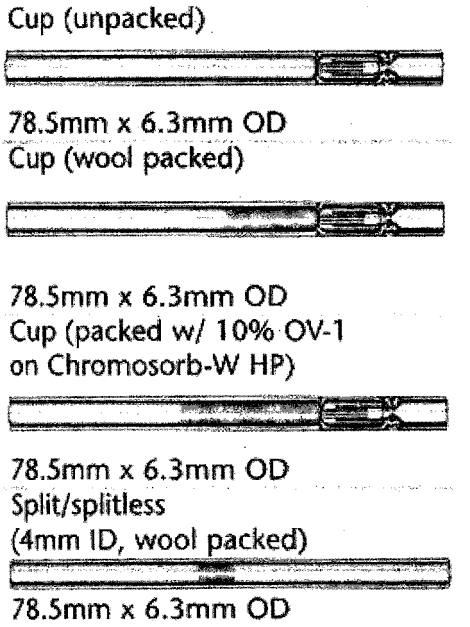


FIGURE 9.5 Glass liner geometries for split injection [adapted from the Supelco Catalog (Supelco, Inc., Bellfonte, PA, 2000)].

silanol groups on the glass, which are the most likely cause of sample component adsorption. For the best quantitative reproducibility, "home brew" silylation of used glass liners is not recommended; deactivated glass liners should be purchased directly from a vendor.

9.3.5 Ferrules and Fittings

Perhaps the biggest advancement in inlet technology has been the replacement of most classical valves and fittings with solid-state, electronically controlled components. Still, care must be taken to use only those ferrules and fittings recommended by the inlet manufacturer and to install them correctly, according to their manufacturers' instructions. With the split inlet, special care should be taken to ensure that the column is inserted the correct distance above the ferrule (an incorrect insertion distance can cause loss of recovery and peak tailing), that the column fitting is not overtightened (which can cause leaks), and that the septum and glass sleeve installation fittings are not overtightened, which can cause these to deform, break, or leak.

9.3.6 Setting the Inlet Temperature

The temperature of a split inlet should be set high enough such that there is enough thermal mass and energy in the inlet to vaporize the injected sample

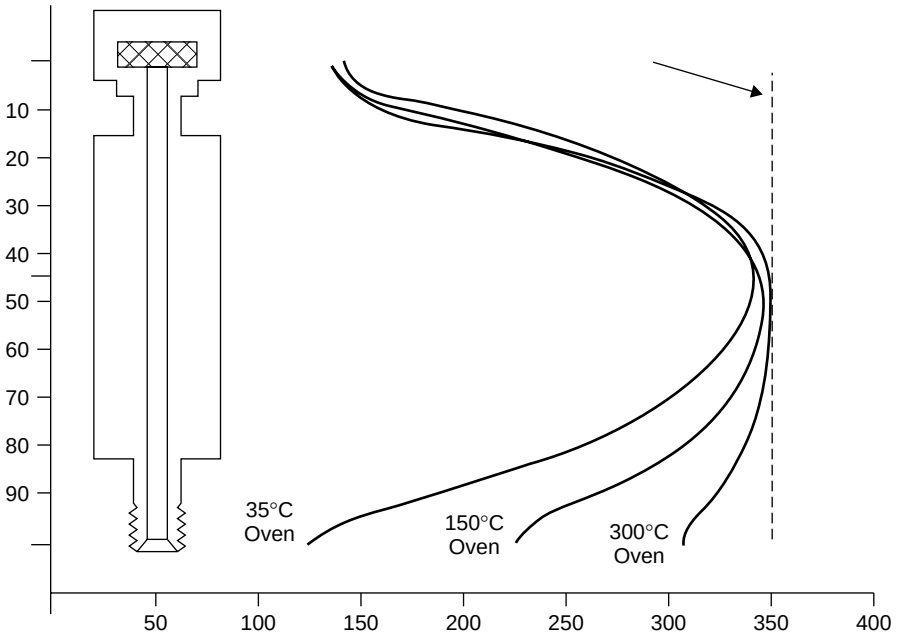


FIGURE 9.6 Inlet temperature profile [length (cm) versus temperature (°C)]. Note the highest temperature at the center of the inlet, with lower temperatures toward the septum and oven wall.

without causing the inlet to cool significantly, but not so high that sample components are decomposed. Often, this must be determined by experimentation by measuring detector signal for the sample at various temperatures. Most analysts begin this at a temperature of 250°C or at a temperature that is used in the literature for the sample or in an existing method. The temperature profile within the inlet should also be considered. Most inlets are heated by electrical resistance, with the heating device located at the midpoint of the glass sleeve. This means that the inlet will be cooler both at the top, near the septum, and at the bottom, near the oven wall and the column head. The temperature profile of a GC inlet is shown in Figure 9.6.

9.3.7 Setting the Flows and Split Ratio

When using a split inlet, there are several flows that provide the exact injected sample amount. The most important of these is the split ratio, which is the ratio of the volumetric flowrate at the split purge vent to the volumetric flowrate in the GC column. Classically, this was measured manually, using a flowmeter to obtain the purge vent flow and by injecting a nonretained substance to obtain the column flowrate. With electronically controlled systems, these values are

measured or calculated directly by the data system, assuming that the column dimensions, temperature, and desired flows are properly entered by the analyst. The actual volume of sample that reaches the column is then estimated by dividing the injected volume by the split ratio, with a higher split ratio giving smaller injected volume and usually narrower peaks. It should be noted that, because many experimental errors are involved, split ratios are meaningful to only two significant digits at the most.

9.3.8 Discrimination and Linearity of Splitting

Perhaps the most vexing problems faced by analysts using the split inlet relate to sample discrimination and nonlinear splitting, both of which cause split injection to produce confusing results. Discrimination results from sample heating that occurs in several locations and results from the inlet temperature and liner geometry and may occur in the high or low end of sample volatility. Nonlinear splitting is the loss of some components, relative to others that may have similar volatility, and is an indication of sample chemistry or reactivity problems. To an extent, discrimination occurs in all heated inlets, due to heating of the syringe needle. Making the injection as rapid as possible, by using a fast autosampler, mitigates this problem. Further, as part of method development, the choice of glass sleeve geometry and the inlet temperature should be optimized. Nonlinear splitting occurs as a result of adsorption of sample components on inlet surfaces or contaminants. Ensuring that the inlet is scrupulously clean and free of debris such as column and septum pieces prevents this. If adsorption is suspected, then inlet components such as the glass sleeve and metal components should be deactivated.

9.4 SPLITLESS INLET

A splitless inlet is based on the same instrumentation as a split inlet. In fact, on nearly all capillary gas chromatographs, the split/splitless inlet combines the capabilities of both and can operate in either “split mode,” to perform a split injection, or “splitless mode,” to perform a splitless injection. The splitless injection process was developed by accident. In 1969 K. Grob, Sr. (9) was performing an analysis using split injection, and failed to open the split purge vent during an injection. Shortly after the injection, the vent was opened. The resulting chromatogram, shown in Figure 9.7, showed large, well-formed peaks for the analytes, when very poor results were expected. As indicated at the beginning of this chapter, over 30 years later, there remains much to understand about splitless injection, which has become the most commonly used injection technique for trace quantitative analysis. The recent (2001) text (3) and the included compact disk (10) provide perhaps the best picture (actual video) of the many mechanisms and effects in splitless injection.

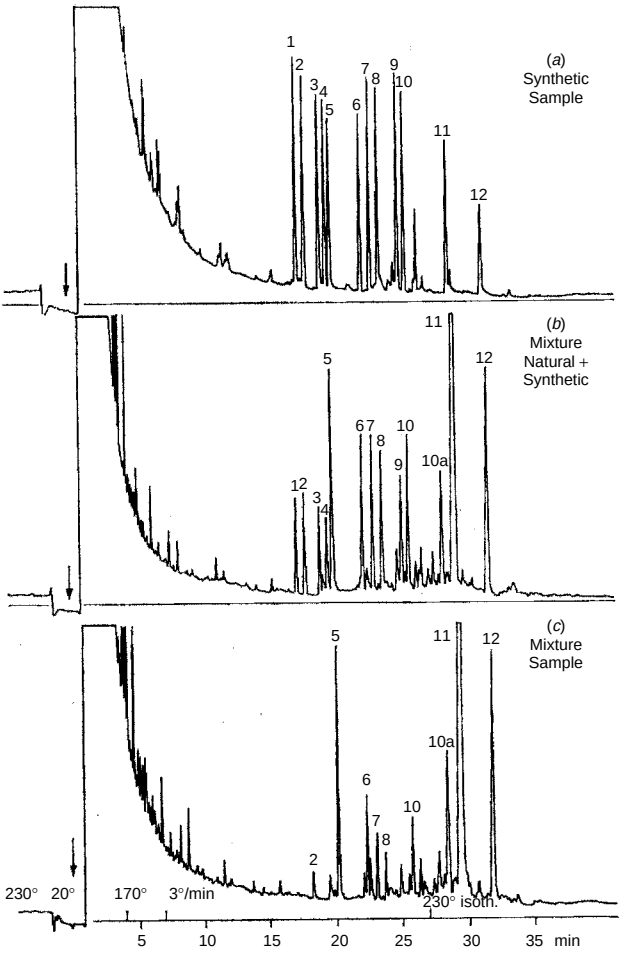
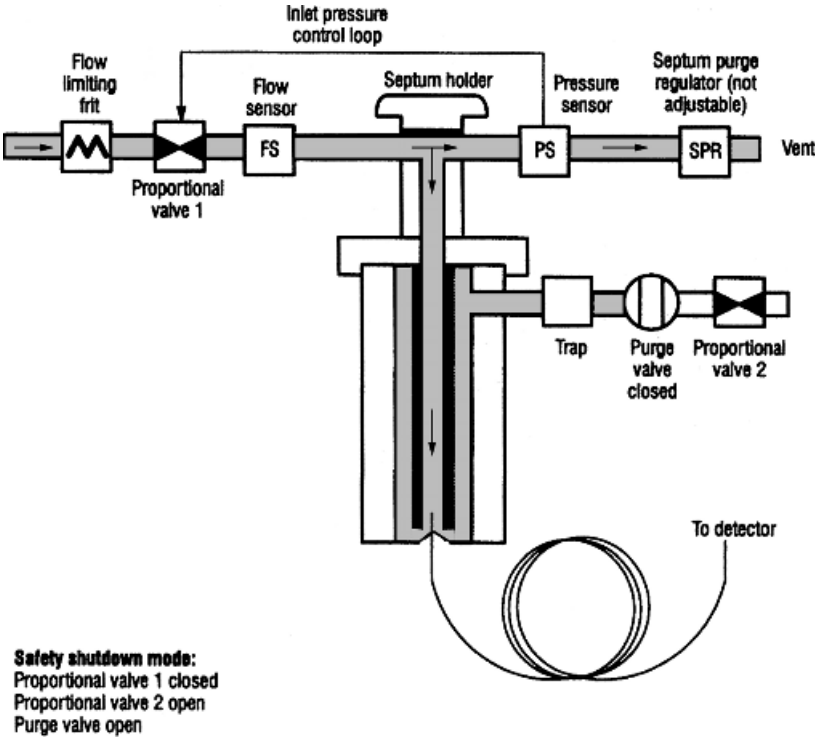


FIGURE 9.7 First application of splitless injection for the analysis of steroids: (a) synthetic sample; (b) mixture of natural and synthetic samples; (c) Natural sample (reprinted with permission from Reference 9, which provides experimental details).

9.4.1 Overview of the Instrumentation

A schematic diagram of a splitless inlet, in both the "purge on" and "purge off" configurations is shown in Figure 9.8. In the "purge on" configuration (Figure 9.8b), the inlet operates as a split inlet. To perform a splitless injection, the purge valve is switched to the OFF position, as shown in the top figure. Since the inlet is backpressure-regulated, the flow is redirected so that the inlet pressure is maintained, which maintains flow through the column, but the volumetric flow through the glass sleeve is greatly reduced. While the purge valve remains off, an injected sample has no place to go from the glass sleeve, but into the column. As in the split inlet, the splitless inlet is heated to ensure sample vaporization



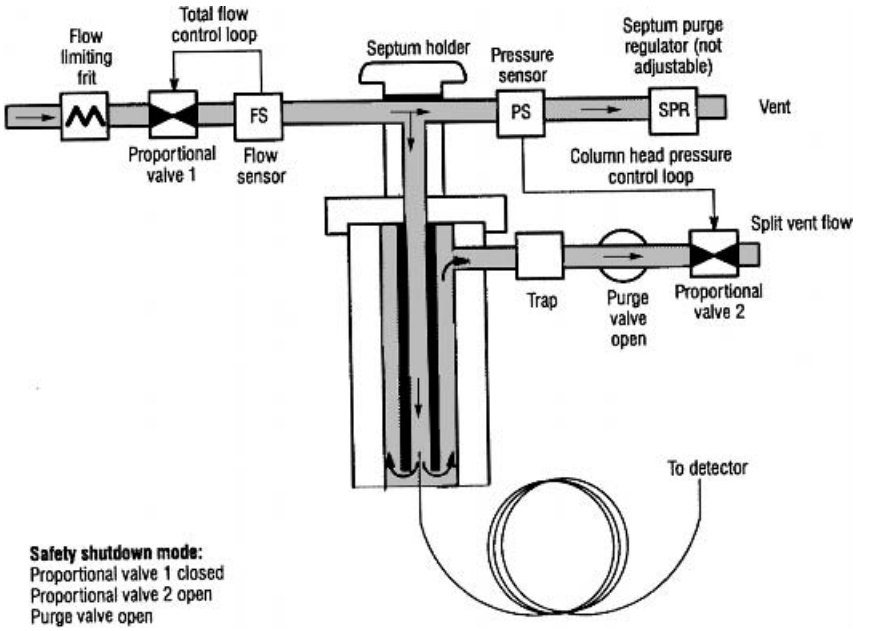
(*a*)

FIGURE 9.8 Diagrams of splitless inlet with (a) purge off and (b) purge on. (Reprinted with permission from *6890 Gas Chromatograph User's Manual*, Agilent Technologies, 1995).

and mixing with the carrier gas. After a period of time, typically 30–45 s, the purge valve is turned to the ON state. In order to maintain the pressure in the inlet, a large flow of carrier gas is passed through the glass sleeve and through the purge vent. There are several factors that contribute to the surprising result that splitless injection, which requires a long time to complete, results in sharp peaks. These require that instrumental conditions, such as the glass sleeve, the inlet temperature, the column temperature and dimensions, injection solvent, and volume and flowrates, be carefully optimized. The septum, fittings, and ferrules used in splitless mode are the same as for split mode. For splitless injection, the glass sleeve is usually a straight tube of approximately 2 mm inside diameter and rarely has any obstructions. As with split injection, deactivated glass wool, packed into the glass sleeve, can assist in vaporization.

9.4.2 Band-Broadening and Band-Focusing Mechanisms

Unlike split injection, which is very rapid, a splitless injection may require up to 1 min for the injection process to complete. It is obvious that splitless injection



(*b*)

FIGURE 9.8 (*Continued*)

would be useless if the injected bands were one minute wide when eluted. Therefore, there must be several mechanisms involved in band broadening and band focusing in splitless injection. There are four major processes that contribute to the eventual sharp bands seen in splitless injection:

1. *Band broadening in time* which arises simply from the time required for the injected material to eject from the inlet and to enter the column.
2. *Band broadening in space* which occurs from the spreading of dissolved analyte in the solvent, as it condenses inside the initial length of the capillary column.

   To mitigate these two causes of band broadening, two band-focusing processes occur (see items 3 and 4, below).
3. *Cold trapping* which occurs for low-volatility analytes. If the initial column temperature is low enough, lower volatility analytes will be frozen in a narrow band at the column head.
4. *Solvent effect focusing* which occurs for higher-volatility analytes. The solvent effects, depicted schematically in Figure 9.9, occur in two ways: (a) the solvent vapor re-condenses rapidly when it reached a column cooled below its boiling point, resulting in a rapid, several-hundredfold reduction in volume, trapping analyte molecules in this flooded zone; and (b) as the carrier gas flows over the flooded zone, it evaporates from the inlet end,

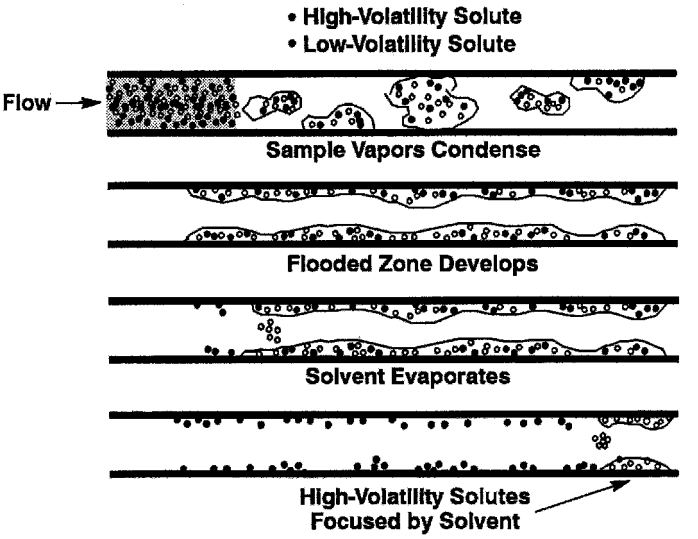


FIGURE 9.9 Solvent focusing occurs in two stages: (1) as the vaporized solvent recondenses from a gas to a liquid and (2) as the solvent slowly evaporates when the oven temperature is increased.

becoming progressively smaller, concentrating the analytes as it evaporates. Therefore, cold trapping can be used to focus low volatility analytes, while solvent effects are used to focus more volatile analytes.

9.4.3 Setting the Temperatures

In order to employ band focusing mechanisms to the best advantages the inlet temperature and the initial column temperature must be set carefully. First, as for split injection, the temperature of a splitless inlet is set high enough to ensure vaporization of the sample without thermally degrading it. The initial column temperature is a much more complex issue. For the solvent effects to be effective in band focusing, the initial column temperature must be low enough to ensure condensation of the solvent following the injection. Typically, this means that the initial temperature must be about 30 or more degrees below the solvent normal boiling point. However, the initial column temperature will have little effect on lower-vapor-pressure analytes. Thicker-film capillary columns can also aid in focusing volatile analytes, by aiding in cold trapping. These values should be carefully optimized as a part of method development.

9.4.4 Setting the Flows and Purge OFF Time

The flow and purge valve settings for splitless injection are relatively straightforward to optimize. The column flow should be set as high as possible during the

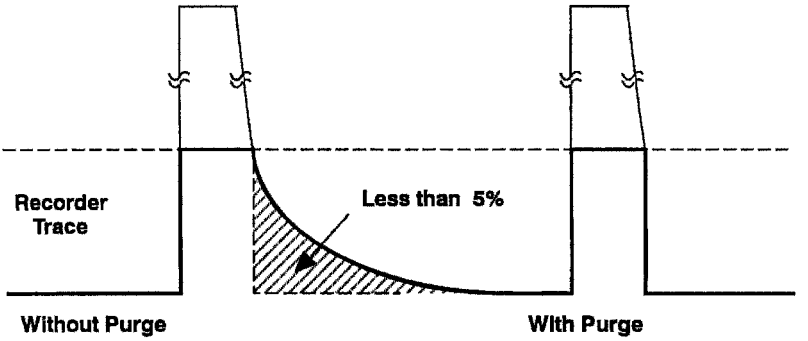


FIGURE 9.10 Schematic showing elimination of the solvent peak "tail" by opening the purge vent. After 95% of the sample has entered the column, the solvent tail is reduced significantly.

injection and then, using the electronic pneumatic controller, it can be reduced to the optimum column flow, as determined by the van Deemter plot. The purge vent flow is generally set to provide a high (100 or so) split ratio when the purge vent is opened, to rapidly "clean" out the inlet and reduce or eliminate the large tail expected on the solvent peak. This is depicted schematically in Figure 9.10. The purge valve is closed at the moment of injection and is later opened. This should occur after about 95% of the solvent has passed. Plotting the peak area of analyte peaks versus the purge valve time can easily optimize this. Generally, this plot will flatten after about 30–60 s, with no benefit, and possible peak shape degradation from longer purge OFF times. Shorter times can be used, but there will be a reduction in the resulting peak heights and areas due to a smaller amount of sample reaching the column. In electronically controlled inlets, a pressure pulse during the injection process can be employed. This is an elevated inlet pressure during the purge OFF time that speeds analyte transfer to the capillary column, resulting in sharper peaks. When the purge is rendered in the ON state, the inlet pressure is reduced to the optimized value for the separation.

9.4.5 Optimization Recommendations

While fully optimizing a splitless inlet can be difficult and perhaps one of the more time-consuming aspects of gas chromatographic method development, there are several general initial steps that can assist in this process. First, the inlet and pneumatics should be properly cleaned and maintained so that leaks and contaminants do not contribute to sample loss or adsorption. For low-volatility analytes, the solvent effects are less important, as most of the band broadening in time can be eliminated by cold trapping. If band broadening in space occurs, then a change in sample solvent or a retention gap (short, typically 5-m-long, piece of deactivated fused-silica tubing prior to the column) can be used. For more volatile analytes, in which all band-broadening mechanisms are in effect, transport time through the inlet should be minimized and the solvent effects

should be employed. A pressure pulse injection, combined with a solvent of polarity similar to that of the column can be used. A thick-film capillary column will also aid in trapping the analytes into sharp bands.

9.5 COOL ON-COLUMN INLET

Of all inlets for capillary gas chromatography, the cool on-column inlet is perhaps the ideal choice for many applications, although its use is somewhat limited. This is the only inlet for capillary gas chromatography that does not require the sample to be injected into an additional chamber and transferred to the capillary column. Further, this is a low-temperature injection, which mitigates potential problems with both syringe needle and inlet discrimination, as well as reactivity of the sample within the inlet. The main advantage of the cool on-column inlet, that the entire sample enters the column, is also the main disadvantage. This means that the analytes and all the interferences will reach the column, which can lead to prohibitive cleaning and maintenance requirements.

9.5.1 Overview of the Instrumentation

A schematic diagram of a cool on-column inlet is shown in Figure 9.11. In many ways, this instrumentation is much simpler than that for split and splitless, as there is no purge vent. The inlet includes a septum, which may or may not have a septum purge. A needle guide ensures that the syringe needle passes easily into the column. It is noted that, for any columns with an inside diameter smaller than 0.53 mm, special syringes with tapered needles are required. There are generally less rugged than standard syringes and are more difficult to handle. The inlet typically has a low thermal mass, to ensure rapid heating and cooling. Most often the temperature of the inlet is ramped along with the column in a temperature program, although the inlet temperature can be controlled separately. Owing to cold injection (little to no discrimination in the syringe needle) and to depositing of the sample directly on the column, cool on-column inlets are by far the most reproducible. With tapered stainless-steel syringe needles, automated injection into 0.53-, 0.32-, and 0.25-mm columns is possible. For smaller inside diameters, special syringes with fused-silica needles that must be operated manually are used. In order to facilitate rapid cooling and low temperatures that maintain the solvent in the liquid phase, most cool on-column inlets are capable of employing cryogenic cooling with carbon dioxide or liquid nitrogen.

9.5.2 Special Considerations

The cool on-column inlet may be used for all types of analyses, but it excels for analytes that are high-boiling, thermally labile, or otherwise reactive in the inlet. Cool on-column is often used as a control when optimizing other techniques, as it does not suffer from discrimination. Analytical sensitivity is usually very

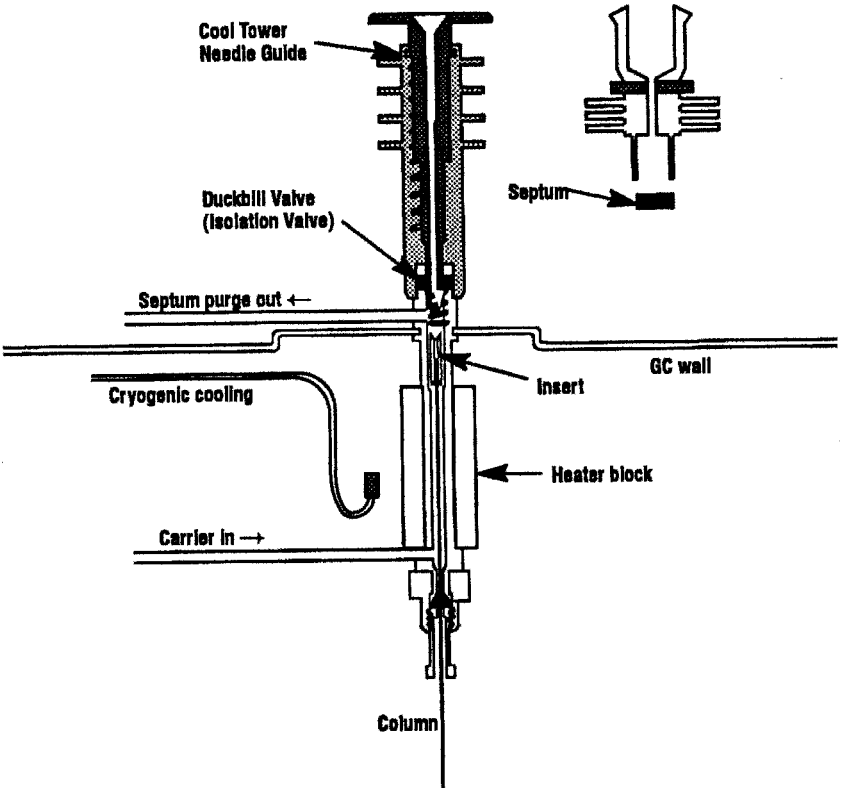


FIGURE 9.11 Schematic of cool on-column inlet. The analytical column or retention gap extends through to the top of the inlet and sample is deposited directly into the column. A "duckbill valve" or a thin septum may be used with either manual injection or an autosampler.

high, and detection limits are usually as good as or better than those for splitless injection. If the inlet has a septum purge, there may be some loss of volatile components, although losses due to solvent vapor expansion in the inlet are not observed because of the cool inlet temperature. The most important disadvantages of cool on-column injection is that the entire sample is injected onto the column and that the special syringes are often difficult to handle. If samples are contaminated or "dirty," this can result in rapid degradation of the column and additional maintenance. Further, as with the splitless inlet, band broadening in space can occur, causing poor peak shapes or split peaks. This can be mitigated by using a retention gap.

9.5.3 Large-Volume Injection

With the addition of a retention gap and a timed vapor exit valve between the retention gap and the analytical column, cool on-column injection may be used

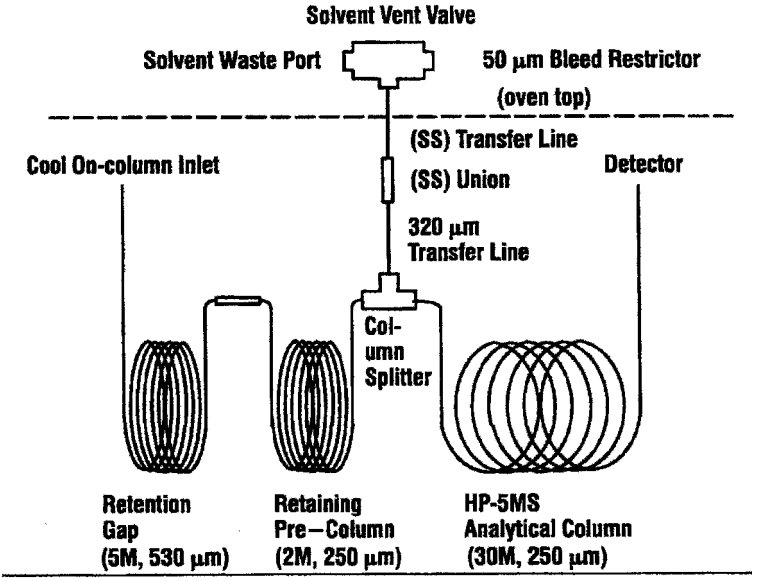


FIGURE 9.12 Schematic of cool on-column inlet with solvent vapor exit for large-volume injection. A retention gap is connected to the inlet. Following the retention gap, a retaining precolumn may be used to enhance retention of volatile analytes. A splitter controls exiting of solvent vapor and directs the analytical sample to the column. (Reprinted with permission from *Solvent Vapor Exit Kit User's Manual*, Agilent Technologies, 1995.).

for the injection of large volumes (up to hundreds of microliters) of sample, with the resulting increase in sensitivity. A schematic diagram of a an on-column system set up for large-volume injection is shown in Figure 9.12. It begins with a retention gap, typically made from deactivated fused-silica tubing of the same inside diameter as the column. This allows room for the large injected solvent volume to condense and expand. Next a retaining precolumn may or may not be used (it is used to assist in retention of more volatile analytes). Following the retaining precolumn, the flow is split between the vapor exit valve and the analytical column. At the moment of injection, the vapor exit valve is opened. As the solvent evaporates in the retention gap, it passes out through the vapor exit. The vapor exit is closed after about 95% of the solvent vapor has exited, leaving the remaining solvent to carry the analytes into the analytical column, where they are focused by solvent effects, as described for splitless injection. Using a large-volume tapered needle syringe, or a 0.53-mm-i.d. retention gap, this technique can be readily automated.

9.6 PROGRAMMED-TEMPERATURE VAPORIZATION INLET

The splitless inlet is most commonly used for trace analysis; however, it has several deficiencies as it is a hot, vaporizing device. In the late 1970s, the

programmed-temperature vaporization (PTV) inlet was developed, based on the splitless inlet, to mitigate these problems. In short, a PTV inlet is a split or splitless inlet that is cool at the moment of injection, then the inlet is temperature programmed rapidly to transfer the injected sample into the column. With a PTV inlet, both split and splitless injections can be performed with the inlet cooled and injection of large sample volumes all at once, or over a period of time, are allowed. This is perhaps the most versatile of all GC inlets.

9.6.1 Overview of the Instrumentation

A schematic diagram of a PTV inlet is shown in Figure 9.13. This is very similar to the splitless inlet, except that it includes a pipe for the introduction of cooling gas (air, carbon dioxide or liquid nitrogen) to allow rapid cooling of the inlet, and in some inlets, an additional purge line. Further, the inlet tube and glass sleeve have a low thermal mass, again to allow rapid heating and cooling, while the split/splitless inlets have a high thermal mass. The glass liners for PTV are often smaller in size and lighter in mass than those used for split and splitless inlets, and they are often packed with inert material, to increase capacity and aid in the

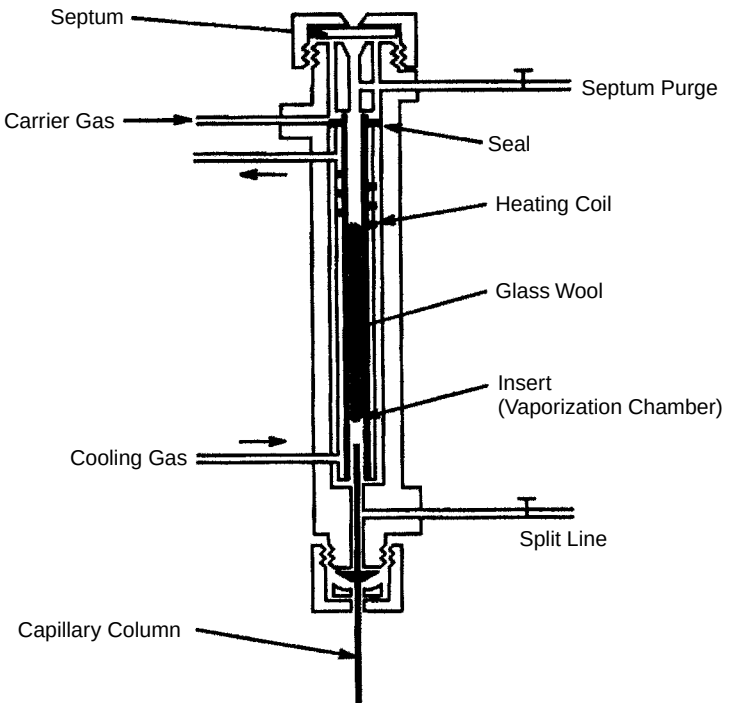


FIGURE 9.13 Schematic Diagram of PTV Inlet. The inlet liner is packed with deactivated glass wool or other inert packing material to hold the liquid sample during vaporization.

introduction of large volume samples. When a sample is injected, it is usually injected into a cooled inlet with a packed liner. The liquid solvent lands on the packing and begins to evaporate, with the solvent vent either open or closed, or programmed, according to the mode of operation, as described below. In all modes, the inlet is heated (up to 16°C/s) to transfer the sample to the capillary column. The inlet can operate in classical hot split and splitless modes, in cool split and splitless modes and in cool split and splitless solvent vent modes for large volume injection.

9.6.2 Modes of Operation

9.6.2.1 Hot Split and Splitless

If the inlet temperature is maintained hot and constant throughout the analysis, the PTV inlet operates in exactly the same manner as a classical split or splitless inlet. These operations are described elsewhere in this chapter.

9.6.2.2 Cold Split and Splitless

For usual liquid sample volumes (0.1 μL to a few microliters) the PTV inlet may operate in a cool split or splitless mode, which can be advantageous over hot split and splitless for thermally labile sample components. At the moment of injection, the inlet is cool enough to ensure that the solvent does not immediately evaporate. The injected sample enters the glass sleeve as a liquid and evaporates as the inlet is rapidly temperature-programmed to a high temperature over a period of seconds. The solvent and sample then transfer to the capillary column in a similar fashion to hot split and splitless. The cool temperature during the injection eliminates syringe needle discrimination and the controlled heating of the sample reduces thermal lability.

9.6.2.3 Cold Splitless Solvent Vent (Large-Volume Injection)

Perhaps the most interesting aspect of PTV injection is the ability to inject large sample volumes using the cold splitless solvent vent technique. In this technique, a large volume (up to 100 μL of most solvents) is injected either all at once or in a series of smaller injections, with the inlet cool and with the solvent vent open. The glass liner usually contains an inert packing to improve capacity, providing a large surface area to accept the large liquid volume. As the solvent evaporates with the vent open, it is ejected through the vent. After about 95% of the solvent is evaporated (this timing can be calculated or determined through method development), the solvent vent is closed and the remainder of the material is transferred to the column in a splitless fashion by temperature programming the inlet. When this transfer is complete, the solvent vent is opened again to clean out the inlet, in the same fashion as splitless. The column is then temperature-programmed. All the band broadening and focusing mechanisms that occur in splitless injection also occur in this technique.

9.6.3 Advantages and Disadvantages

Because it is a cold injection technique that also employs a glass sleeve, PTV injection offers several advantages. First, it is ideal for thermally labile samples and samples with a wide boiling range. PTV inlets can be programmed to temperatures higher than the usual column temperatures, allowing injection of compounds that might not pass through classical split and splitless inlets. There are a wide variety of glass sleeve geometries to allow for all of the different modes of operation. The PTV inlet is essentially five inlets in a single unit, offering great versatility. This versatility is also the main disadvantage of the PTV inlet. It is, by far, the most complex of the inlets for gas chromatography. Method development is more complex, with a number of additional parameters to optimize. Method development examples for the PTV inlet are provided in the manual by Janssen (6). Because of this additional complexity, the pneumatics and control software are also more complex, increasing the capital cost of the instrument. Finally, since it is not as common as split, splitless, or on-column injection, there is less literature and application notes available to assist in method development and optimization.

9.7 SUMMARY AND CONCLUSIONS

The inlet is perhaps the most complex part of a gas chromatograph. It not only provides a means for introducing samples into the column but also controls all the carrier-gas flow. There are a number of inlets available for gas chromatography, and each has appropriate samples. Optimizing the injection process remains generally the most difficult aspect of method development in gas chromatography, especially in trace quantitative analysis. While there is a great body of literature, there are relatively few conclusions about method optimization that can be applied to all samples and sample types.

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CHAPTER TEN

Gas Management Systems for Gas Chromatography

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10.1 INTRODUCTION

When you think of installing a gas chromatograph, you need to consider the entire system in order to run the new chromatograph(s) to peak efficiency. This means thinking of all aspects of the installation. The information in this chapter will help you design your gas management system wisely and obtain efficient performance from one or many gas chromatographs in a laboratory.

Three major sections make up this chapter. The first section starts with the consideration of the choices to be made with carrier-gas selection, and how that selection influences your chromatography. The second section considers the choice of external items needed to configure a system. These items deal with electrical power and grounding, choice of regulators, tubing choices and selection of external valves and fittings. The third section deals with the design of a system for a simple gas chromatograph installation to very complex multi chromatographic systems. Provided in the third section are diagrams specific for installing gas delivery systems for 1, 2–4, or 5–20 or more gas chromatographs. This chapter provides recommendations that should be used along with the directions in your instrument manual. These directions should be read before you attempt any installation of your gas chromatograph(s).

10.2 CHOICE AND EFFECTS OF MOBILE-PHASES SELECTION

10.2.1 Factors Governing Choice of Carrier Gas

The detectors you will use will dictate your first choice of gases and later other factors defined in your analysis methods will finalize the carrier-gas selection process (1). Table 10.1 lists popular detectors for a gas chromatograph and the gases used with each detector. Table 10.2 provides guidelines for a chromatograph equipped with two flame ionization detectors that requires carrier gas, fuel, and an oxidant gas. Also provided are guidelines for makeup gas and other gas flows typical for these types of systems (2,3). Consult your instruction manual for specific gas requirements for your instruments.

To start the selection process, first develop a list of the types of detectors you anticipate using. From this list, choose the gases that you will need. The

TABLE 10.1 Gases Used with Commonly Used Detectors

Detector	Carrier Gas	Fuel Gas	Make-up Gas
ECD	Nitrogen, argon–5% methane	None	Nitrogen, argon–5% methane
ECD	Helium	None	Argon–5% methane
ELCD[a]	Helium, hydrogen	Hydrogen	None
FID	Helium, hydrogen, nitrogen	Air + hydrogen	Nitrogen, helium, hydrogen
FPD	Nitrogen, helium	Air + hydrogen	Same as carrier gas
HID	Helium	None	Helium
NPD	Helium, nitrogen, hydrogen	Air + hydrogen	Helium
PID	Helium, hydrogen, nitrogen	None	Nitrogen, helium
TCD	Helium, hydrogen	None	Same as carrier gas

[a] Electrolytic (Hall) conductivity detector.

TABLE 10.2 Gas Requirements of Gas Chromatographic Systems with Flame-Type Detectors[a]

Gas	Flow/Column (mL/min)	Total (mL/min)
Dual Packed-Column Gas Chromatograph with Two Detectors		
Carrier	20–60	40–120
Air (fuel)	350	700
Hydrogen (fuel)	30	60
Dual Capillary Gas Chromatograph with Splitters and Two Detectors		
Carrier	0.5–10	1–20
Split	100	200
Septum purge	4	8
Total	105–114	209–228
Makeup gas[b]	30	60
Air (fuel)	350	700
Hydrogen (fuel)	30	60

[a] Other detectors may not require fuel gases (see Table 10.1).
[b] Often, but not always, the same gas as the carrier.

list should include for each unit the carrier-, fuel-, and makeup-gas needs. You will need a separate line for each gas. A general-purpose gas system for a laboratory, with several gas chromatographs with different detectors, typically has five dedicated lines: helium, nitrogen, hydrogen, air, and actuator (usually inexpensive compressed air), plus an auxiliary line. The auxiliary line anticipates a future need for a special carrier gas, such as argon or argon/methane or hydrogen/helium blends. Do not use carrier, fuel, or makeup gas as an actuation gas.

Device actuation will temporarily disturb the gas supply to the gas chromatograph and affect its performance. The quality of the gas used for valve actuation is not demanding, so there is no need to use high-purity gas for this purpose. Make sure that the actuation gas is oil and particle-free to obtain the best long-term performance from the actuation equipment.

10.2.2 Choice of Carrier Gas Using van Deemter Plots

The choice of carrier gas will also be dependent on the type of columns you will use. For capillary columns the two most popular carrier gases are helium and hydrogen. When using packed columns, most analysts choose between nitrogen and helium. A van Deemter equation allows the comparison of efficiencies obtainable with carrier gases (4–9). The van Deemter equation (Equation 10.1) expresses the extent a component band spreads as it passes through the column in terms of physical constants and the velocity of the mobile phase:

$$\text{HETP} = \frac{A + B}{\mu + C\ \mu} \tag{10.1}$$

where HETP = height equivalent to a theoretical plate
μ = linear velocity of carrier gas (mobile phase)
A = a constant that accounts for the effects of "eddy" diffusion in the column (the A term is not used with capillary columns because there is only one flow path and no packing material in a capillary column)
B = a constant that accounts for the effect of molecular diffusion of the vapor in the direction of the column axis
C = a constant proportional to the resistance of the column packing to mass transfer of solute through it

Linear velocity is

$$\mu = L/t_{\text{M}} \tag{10.2}$$

where L = length of the column in centimeters
t_{M} = retention time in seconds of a nonretained compound, which is typically methane

One determines the HETP values experimentally at various carrier-gas velocities and plots these values for each type of carrier gas. See Figure 10.1 for a plot of typical HETP versus linear velocity values for various carrier gases. Figure 10.1 shows the lowest HETP value for nitrogen; therefore, it is the most efficient.

The minimum value HETP is not the only consideration used in choosing a carrier gas. When considering the best gas for our analysis, we want to consider more than just the minimum HETP value. Figure 10.2 shows a theoretical plot of

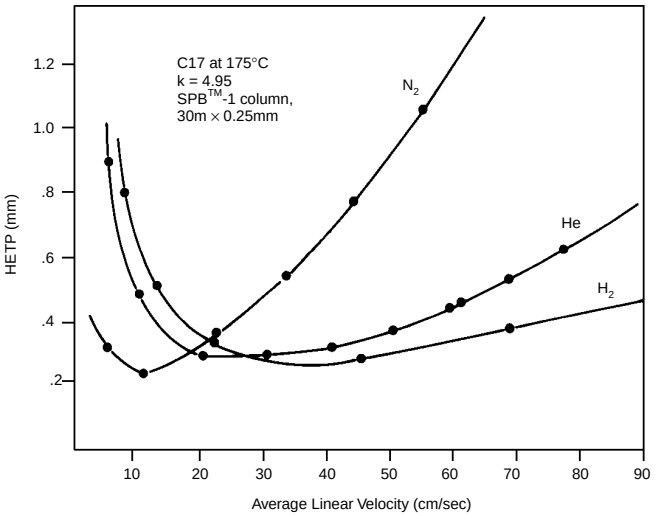


FIGURE 10.1 Typical plot of HETP versus linear velocity.

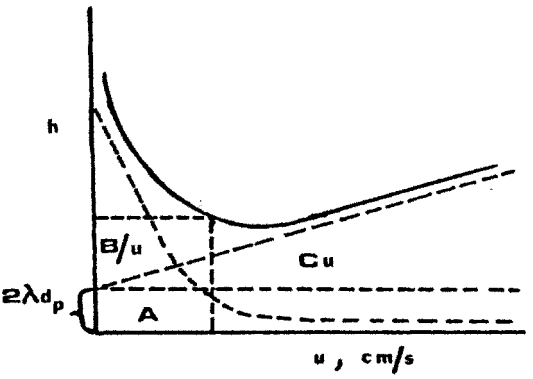


FIGURE 10.2 Van Deemter plot; showing change in *h* versus linear gas velocity.

the van Deemter equation. This plot shows the *A* contribution to eddy diffusion, the *B* contribution of molecular diffusion, and the *C* term of resistance to mass transfer. The slope of the HETP versus the linear velocity often affects our choice of carrier gas. You should pick a carrier gas that has a slope that shows the least reduction in HETP per increasing linear gas rate. This gives you the greatest flexibility in ranges of flows without noticeable loss in efficiency. This slope differs for the three gases. The initial indication is that although nitrogen has the most favorable HETP, hydrogen exhibits the least variation in HETP values over the widest ranges of flows. Hydrogen's HETP optimum starts at flows at least 4 times higher than nitrogen and thus provides the shortest analysis time or fastest runtimes when

run at its optimum or higher linear gas rates. Hydrogen often becomes the gas of choice with very long columns where runtime reduction is paramount.

Safety and cost of carrier gases play a role in carrier-gas selection. Our concern for safety deals with the fact that columns do break in ovens. Many analysts are concerned with using hydrogen as a mobile phase because of this fact. In the United States, helium is inexpensive and readily available in high purity. For this reason, in the United States, the most common choice of carrier gas for capillary columns is helium.

10.2.3 Viscosity Effects Causing Possible Problems during Temperature Programming

The viscosity of the mobile phase will change as you increase oven temperature (11) (see Figure 10.3). Several gas chromatographic detectors are sensitive to changes in the flowrate that are a result of the mobile-phase viscosity changing. One observed result of this effect is the displacement of your baseline during temperature programming. This can result in displacement of the baseline either positively or negatively depending on the gas. The other important factor is that the response of the detector is affected by flow changes because of temperatures effect on viscosity. To avoid this problem we look for a gas with the smallest ratio of viscosity to diffusion coefficient. Rating the gases on the basis of this ratio gives us, from best to worse, hydrogen, helium, and nitrogen. Many of the

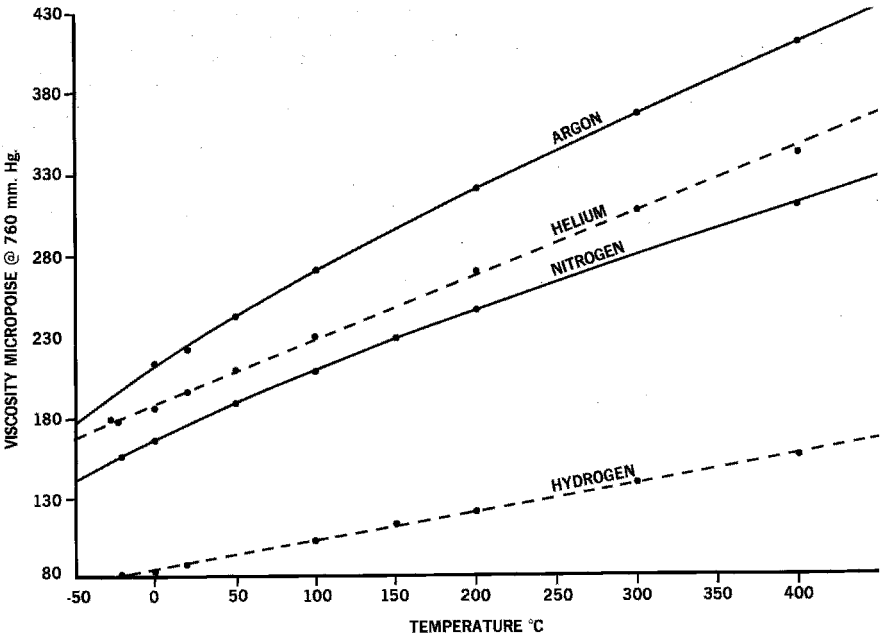


FIGURE 10.3 Effect of temperature on viscosity of four common carrier gases.

modern gas chromatographic systems compensate for these effects by the use of backpressure regulation combined with flow control that is computer-controlled to adjust for the viscosity change of the carrier gas with temperature change. However, all do not. You will find this problem prevalent with TCD detectors and chromatographic systems using valves for sample introduction.

You should generate your own van Deemter plot for the analysis temperature you intend to use and determine the optimum linear velocity best for your analysis. Most people set their linear velocity at the initial oven temperature. As the temperature rises during a temperature-programmed run, the optimum linear velocity will change. For many separations, you do not need to consider optimization, as it is not critical to the separation and the change in optimum flow velocity is small.

For some methods, you may have the problem of establishing a run condition that will separate a critical pair of compounds appearing in the middle or late in a chromatogram during a temperature program run. For best separating capability for this pair of compounds, you determine the temperature at which they elute, and adjust your linear velocity to an optimum value for that temperature. You should consider optimizing the linear gas rate before selecting options such as trying a column with a different stationary phase.

10.2.4 Consideration of Flow Devices — Positive Attributes of Modern Electronic Pressure Control Devices

Most new chromatographs have electronic pressure control (EPC) devices. EPC flow control devices use mass flow controllers; back and forward pressure controls and the combination of these devices control the flow during an analysis (12). You should consider the type and limitations of your EPC system for optimum use of your equipment. Most EPC systems differ in configuration according to the type of column or injection system. Split/splitless capillary inlets use backpressure regulators combined with mass flow controllers. Typically, you see forward pressure control systems with inlets that use a septum purge flow. Auxiliary gases and packed inlets also use forward pressure control. Packed-column gas chromatographs typically use mass flow control, or pressure control may be utilized with the use of sample or switching valve.

EPC compensates for the pressure surge seen during injection that typically caused baseline disturbances with the old mass flow controllers. The use of flow or pressure programming added very new dimensions to the tools used to optimize a separation and to reduce analysis time. It also compensates for changes in viscosity of carrier gases during temperature program runs. The use of EPC used with flow-sensitive detectors has shown reduction in baseline drift.

10.2.5 Mass Flow Controllers for Packed Columns

Most new chromatographs have mass flow controllers. These devices provide excellent control of carrier-gas flow. When using a mass flow controller you will need to do periodic calibration. Check your calibration at least once a year.

Most EPC systems are set up for capillary columns and do not provide correct flows for packed columns. They do not consider particle expansion in packed or PLOT columns. Nor do they compensate for particle rearrangement, resulting in changes in the backpressure due to resettling of the packing during the cooling of a packed column. You use mass flow controllers to set and maintain flows, but you need to verify these flows by manual means when there are temperature or pressure changes introduced into the system. Since the packing density and the amount of packing material change from packed column to packed column, it is necessary to manually check flows between columns. Also, packed columns using carbon, porous polymers, or diatomite packings, because of their different coefficients of expansion, react differently to temperature changes. This means that mass flow controllers may not be able to provide constant flow during temperature programming of packed columns.

The rapid decompression of a packed or a porous-layer open tubular (PLOT) column can ruin the column. The sudden pressure reduction can dislodge packing from the walls of a PLOT column and with a packed column cause the packing to shift within the column. You should allow a slow reduction of pressure at the end of a pressure-programmed run to avoid damage to the column.

10.2.6 Pressure Control for Capillary Problems

The use of temperature programming to speed up an analysis has its limits. As you increase temperature, the potential for column bleed increases. Even with the improvement of capillary columns toward low-bleed columns, the potential for bleed exists. With increased temperatures comes increased column bleed that often results in shorter column life. Certainly, the relationship between higher temperature runs and shorter column life has been a given for years. One solution to this problem is to combine temperature programming with electronic pressure control (EPC). You can, at the same time, temperature/pressure-program both column and injectors to speed up an analysis. Many labs have developed methods that involve simultaneous temperature programming with pressure programming.

10.2.7 Proper Measurement of Flowrates with Packed Columns and Appropriate Devices

You can measure the flow through a column in many ways. With packed columns, the use of electronic measurement devices is becoming increasingly common, although many people still use soap-bubble meters to measure flow. The use of bubble meters has been common practice for years. However, there are technical problems with the use of soap-bubble meters. Water/soap meters have errors with water negatively influencing the flowrate, causing apparent higher readings of the magnitude of 2–4% of error. Gas diffusion through soap films introduce errors resulting in lower than true readings. The restrictions seen in the bubble meters may cause flow errors and fluctuations in the flows. You can expect to see small differences between electronic measuring devices and bubble meters.

10.2.8 Measurement of Linear Velocity with Capillary Columns

With capillary columns, we have many different flows to measure with extremely large differences in the range of flows. Capillary columns may have flows lower than 1 mL/min, yet have septum purge flows in the 2–5 mL range and splitter vent flows in the 100–300 mL range. It is preferred to measure capillary column flows in terms of linear gas rates. It is acceptable to measure the other flows with electronically controlled flow measuring devices or soap-bubble meters. Since the split ratio for an analysis is determined by dividing the flow of the column into the vent flow volume from the splitter, we normally do both of these flows electronically. Any other flows that are to be measured are not critical and either manually or electronic measurement is acceptable.

10.3 CARRIER-GAS PURITY, CONNECTIONS, TUBING, AND RELATED ISSUES

10.3.1 Basic Installation Concerns

10.3.1.1 Power Requirements

Whether you are planning a single gas chromatograph, a bench of gas chromatographs, or a gas chromatograph laboratory system, we recommend that you have a qualified electrician review your power needs and recommend a suitable power system. Be ready to provide an estimate of the total power requirements for all gas chromatographs and associated equipment. A typical gas chromatograph consumes approximately 2100 W and requires a 15–20-A *dedicated, grounded* outlet. This avoids transient signals from other sources (elevator or machinery motors, vending machines, fluorescent lights, etc.). Add to this the power needs of the integrator, plus peripheral equipment that you anticipate using (autosampler, thermal desorber, pyrolizer, etc.). The integrator or data system should be on the same outlet or circuit as the gas chromatograph from which it is acquiring data. This will help prevent ground loop currents and reduce baseline noise. Equipment requiring electric actuation, such as electric valve actuators, should be on a separate line. Be sure that the outlets will be located near the instruments, and will be in sufficient number to meet current and future needs. Never use an extension cord of any type or rating to connect a gas chromatograph.

It is important to have isolated and insulated electrical grounding for these instruments. In many buildings, you find that water lines and other sources of grounding do not provide adequate grounding. Maximum allowable line noise on a ground line is 3 V (RMS), from 30 Hz to 50 kHz. We also recommend incorporating surge protection in these lines.

10.3.1.2 Gas Choices

The gases you will need for your chromatograph are a function of the types of detectors you will use and the particulars of your analyses. Table 10.1 lists typical gas chromatograph detectors and the gases used with each. The preference

for one carrier gas versus another also can differ from one analysis to another. A chromatograph equipped with two typical flame-type detectors will require carrier, fuel, oxidant, and, for some analyses, makeup gas, in the amounts shown in Table 10.2. Consult your instruction manual for specific gas requirements for your instrument.

List the types of detectors you anticipate using, and their requirements for carrier, fuel, air, and makeup gases. You will need a separate line for each gas. A general-purpose system with several types of detectors typically has five dedicated lines: helium, nitrogen, hydrogen, air, and actuator (usually inexpensive compressed air), plus an auxiliary line. The auxiliary line anticipates a future need for a special gas, such as argon or argon/methane or hydrogen/helium blends.

Do not use carrier, fuel, or makeup gas as an actuation gas. Device actuation will temporarily disturb the gas supply to the gas chromatograph and affect its performance. In addition, the quality of the gas used for valve actuation is not demanding, so there is no need to use high-purity gas for this purpose. On the other hand, the actuation gas must be oil- and particle-free, for long-term best performance from the actuation equipment.

10.3.1.3 Cylinders or Generators?

Most labs traditionally have used compressed-gas cylinders, but today, primarily for safety and practicality, gas generators are becoming increasingly common. If you choose to use cylinders or tanks, your gas supplier can help you determine the sizes and numbers of cylinders you will need, and can help you design the plumbing for your system. Your supplier can provide cradles for six or eight cylinders, already manifolded. A single line connects the cradle to your house line. Depending on the size and needs of your system, you can use Dewars, bulk tanks, or tube trailers as sources of compressed gas.

Gas generators can greatly simplify plumbing systems and eliminate the need for handling high-pressure and flammable materials. Because these compact units typically can be located very near the instruments they serve, they eliminate the need for long gas lines and cylinders mounted in hallways. Compact, high-purity, worry-free, and safe generators of nitrogen, air, and hydrogen are available. Hydrogen generators, in particular, provide important safety advantages. Relative to cylinders, the total amount of stored gas is small, and pressures are much lower. This significantly reduces the risk of explosion. Safety devices internal to most generators shut down the units when the pressure surges or suddenly drops. Maintenance time spent on generators is less than that spent on changing cylinders.

There are a few negative aspects to the use of gas generators. They do require semiannual maintenance. Filters and purifiers must be changed and moisture dropout device checked for retained water. Hydrogen generators have many built in safety devices. Trying to restart a hydrogen generator sometimes can be time-consuming because of safety systems required. When starting up a hydrogen generator if it cannot reach pressure in a minute or so, it will turn off. This is a safety feature designed to shut off a unit in case of a line rupture. Startup

of a large system (several gas chromatographic units) may involve starting the hydrogen generator 10–20 times until sufficient pressure builds up. Many of the manufactures of hydrogen generators recommend that you have a hydrogen cylinder available to help pressurize the system. After the cylinder is used to pressurize the system, the hydrogen generator is switched on and the cylinder turned off.

Nitrogen and air generators are dependent on house air systems or standalone air compressors. House air systems do go down occasionally and are seldom monitored for their moisture and hydrocarbon content. If a nitrogen or air generator becomes oil saturated you face replacement of the entire unit. Repair of gas generators can get very expensive very quickly.

Hydrogen Generator Hydrogen generators electrolytically break water down into hydrogen and oxygen. Hydrogen will receive further purification though palladium-permeable filters, and the oxygen is vented (13). When used with a downstream water-trapping system, such as a molecular sieve trap and an indicating trap in series, a hydrogen generator will provide GC-quality hydrogen for both carrier-gas and detector fuel use. New models of hydrogen generators produce hydrogen at a purity of >99.99999%, and internal purifiers eliminate the need for additional downstream purification.

To determine how many hydrogen generators you will need, calculate expected flow needs according to the number of gas chromatographs and the types of detectors and other equipment you will be using (Tables 10.1 and 10.2) or specific flow information from your instrument manuals). Once you know how much hydrogen you will need, you can determine which model or models will meet that need from Table 10.3. It is always best not to run at full capacity and to purchase a unit with excess capacity.

Hydrogen generators require deionized water of 500,000-Ω/cm resistance, or greater, or a sodium hydroxide solution. A major safety advantage for hydrogen generators is that at any time, the total volume of gas in the unit is small, and the pressure is low. Most units have a pressure-relief valve, set for a pressure slightly above the normal operating pressure. Other safety devices within the generator also ensure that pressures cannot exceed the specified maximum, and shut down the unit if the pressure suddenly drops. You should see to the proper

TABLE 10.3 Hydrogen Output of Hydrogen Generators

Flow (mL/min)	Maximum Pressure (psig)
0–125	90 (6.3 bar)
0–250	90 (6.3 bar)
0–500	90 (6.3 bar)
0–1200	100 (6.3 bar)

plumbing of the safety vent on the back of any hydrogen generator to ensure safe venting (14).

Air Generator An air generator is, in fact, a sophisticated air purifier. Typically, the source is house air from a compressor or low-grade compressed-air cylinders. When properly installed (Figure 10.4), a zero-air generator will provide air at a purity exceeding the quality demands of your gas chromatograph.

As with hydrogen, determine your air requirements from Tables 10.1 and 10.2 and your instrument manuals. One unit can provide ultra-high-purity air to multiple detectors. Be sure to plan for extra capacity, even if it means buying an extra unit—*do not* run generators at 100% of capacity.

You should provide the incoming compressed air at less than 200 ppm total hydrocarbons and particles smaller than 7 μm. Compressed air plumbing systems can contain rust, oils, and condensed liquids. To remove oils, sulfur-containing compounds, and halocarbons from the source air, install an oil-removing (coalescing) filter, a vapor-removing filter, and a hydrocarbon trap before the generator (Figure 10.4). If your plant air system does not sufficiently dry the air, install a molecular sieve drying tube between the coalescing filter and the generator inlet. A coalescing filter within the generator removes the last contaminants from the air.

A zero-air generator operates best when supplied with compressed air at 125 psig (8.8 bar) or less and a flow of 2500 mL/min or less. Upstream from the hydrocarbon trap, install a single-stage pressure regulator with a pressure gauge that will show operating pressure to at least 125 psig (8.8 bar). We suggest a gauge that will show pressures to 200 psig (14 bar).

Nitrogen Generator There are two approaches to generating pure nitrogen. In one approach, compressed air passes across a semipermeable membrane that

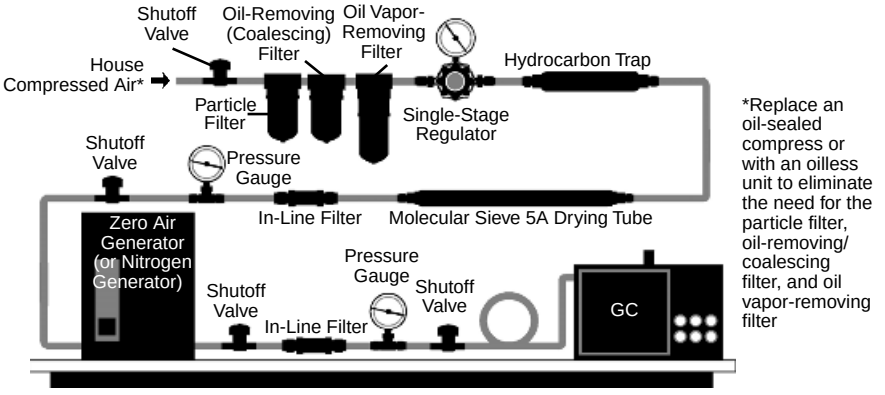


FIGURE 10.4 Filters and traps ensure high-quality incoming air for a zero-air generator (or for a nitrogen generator) [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

allows nitrogen to permeate and almost completely impedes the transfer of other air components and contaminants. At high flowrates, oxygen does break through. The second approach is a two-stage process that uses two adsorption beds of carbon molecular sieves. Air enters the front of the cylinder, and as it passes through the cylinder, it is separated into oxygen and nitrogen as it passes through the carbon molecule sieve. The nitrogen is allowed to move out of the first bed until the oxygen nears the end of the carbon bed. At this point, the flow is reversed in the cylinder and nitrogen coming off the second cylinder is allowed to purge the first cylinder. This process is called *pressure swing adsorption*. Alternatively, both cylinders are charged with air and provide nitrogen. The nitrogen is used to both purge and reverse flow the cylinder being cleaned and to provide nitrogen for analytical purposes. Both methods work well—we recommend selecting a unit based on the flow needs and purity requirements of the applications you intend to use (remember to allow for future changes).

Additional purification is still recommended. Passing the nitrogen leaving the generator through supplemental purifiers can reduce the oxygen, carbon dioxide, and water levels in the nitrogen even lower than the sub-parts-per-million levels supplied by the generator.

Calculate the nitrogen needs of your system, based on the number of chromatographs and types of detectors you plan to use (Tables 10.1 and 10.2 and your instrument manuals; see also Table 10.4 for airflow needs). Be sure to plan for extra capacity—we do not recommend long-term operation of any gas generator at full capacity. To obtain the best performance from the nitrogen generator, remove water, dirt, rust, and oils from the incoming compressed air in the same manner as for a zero-air generator (Figure 10.4).

Be aware of the flow needs of the gas generator(s) that you will be using. With air generators there is an almost 1–1 ratio of incoming gas flow to product gas; there is almost no flow loss. However, with nitrogen generators this is not the case. Most of these units, independent of the purification approach (semipermeable membrane or contaminant adsorption) require large quantities of input air to produce the desired output flow.

Compressors Look critically at the source of your compressed air. Older facilities typically have oil-sealed compressors. The longer and harder they run to meet your gas needs, the hotter they become. This leads to oil and water vapors in

TABLE 10.4 AirFlow Need versus Output of Nitrogen Generators

Nitrogen Generator	Air Consumption (L/min)	Nitrogen Delivered (L/min)	Stated Purity[a] L(%)
Air Products	75	1	99.99999
Whatman			
Model 75	102	1.9–36.8	95–99.5
Model 76	42	0.5–2	99.99–99.9995

[a] As flow is increased, purity is reduced.

the airstream. You can make either of two choices: (1) you can install a series of special filters to reduce the hydrocarbon level in air leaving the compressor to less than 100 ppm (particle filter, oil-removing/coalescing filter, and oil vapor-removing filter, as shown in Figure 10.4), or (2) you can replace the compressor with an oilless unit. Oilless compressors typically use Teflon seals and do not use oil in any way.

Most air compressors have built-in water vapor traps, but the heat generated by the unit can cause significant amounts of water vapor to be present in the air produced. A water vapor trap downstream from the hydrocarbon trap will reduce the water content in the air (Figure 10.4). Depending on whether the compressor is oil-sealed or oilless, the quantity of hydrocarbons will vary greatly. Even oilless compressors can allow hydrocarbon levels that should cause concern. (The location of the air intake for the compressor is very important in determining hydrocarbon levels).

Cylinder Safety If you plan to use compressed gas cylinders, safety should be a primary concern. A typical cylinder for analytical instruments has a pressure of 2000–3000 psig (140–210 bar) on delivery. A rupture at a cylinder valve causes rapid depressurization and can cause serious injury or structural damage to a lab (15).

Store cylinders in a secluded but easily accessible location. Avoid humid places where rust can form on the caps or cylinders, and locations heated by oven exhaust. It is a good idea to establish extra tiedown storage sites near the supply cylinder. This is an excellent way to store safely the extra cylinder during the changing process. Bolt cylinder brackets to a wall or bench. Brackets with screw clamps work but can become loose with long-term use. Wall-mounted brackets with 1–3-cylinder capacity are available. A properly secured cylinder cart is a safe alternative.

Always consider safety when changing cylinders or regulators. Do not move cylinders unless you have a properly equipped cylinder cart with chains to secure the cylinders in place. Never roll a cylinder or move a cylinder with the cap off. Never change a cylinder without safety equipment, including eye protection and gloves. When changing cylinders, remove the expended cylinder (label it "empty"), place it on the cart, and chain it in place, *then* remove the new cylinder from the cart and install it. *First* secure the new cylinder in place, *and then* remove the cap. If the cap will not screw off, do not try to force it. Do not place any object inside the holes in the cap except a tool designed specifically for that purpose. Return a cylinder with a wrong-size cap to the manufacturer properly marked with the problem.

After removing the cap from the new cylinder, inspect the fitting seat. Remove any dirt you observe—it could keep the fitting from sealing properly, or it could be forced into your system. Carefully screw the regulator onto the cylinder and tighten with the proper-sized wrench. Turn fully counterclockwise (valve closed position) the downstream pressure control knob on the regulator. (*Caution*: Using both hands, open the cylinder valve while standing to the side of cylinder. Never

face the gauge(s) when opening a cylinder. Bourdon tubes in pressure gauges can rupture with enough force to cause serious injury.) Slowly open the main regulator valve, then slowly open the downstream pressure control knob, and reestablish the proper line pressure. Be sure to record the pressure on or near the pressure regulator before changing a cylinder.

Use an electronic leak detector (*never a liquid*) to test for leaks (see Section 10.4.1.1, section on testing for leaks). If there are no leaks, open the shutoff valve separating the cylinder and regulator from the rest of the system. If you find leaks between the cylinder and the system, close the cylinder valve (for the correct procedure for relieving pressure in a two-stage regulator, refer to "Additional Comments on Regulators" section, below). Unscrew the fitting, and be sure there is no dirt on the fitting or cylinder seat. If there is no dirt on the fitting and the leak persists, you may need a new fitting, or you may have damage to the seat of the fitting in the cylinder valve. If there is damage to the fitting or the seat, using Teflon tape on the fitting will not work—the sealing point is at the end of the fitting, not on the threads (Figure 10.5).

[*Note*: For detailed information on safe handling of cylinders and regulators, refer to *Safety Measures for Pressure Reducing Regulators* (order from Air Products and Chemicals, Inc., 7201 Hamilton Blvd., Allentown, PA 18195-1501, USA).]

When to Change a Cylinder Most gas suppliers request a minimum residual pressure of 25 psig (1.8 bar) in a cylinder. If no pressure is present, they must specially clean and prepare the cylinder before repressurization. Also, be aware that as the pressure decreases in a cylinder, the concentrations of contaminants in the gas will increase because they can more easily pass from the liquid state to the gaseous state. This is especially true of water—there will be a much higher concentration of water in gas delivered from an almost empty cylinder than in gas from a full cylinder. For this reason, we recommend changing carrier-gas cylinders when the pressure is 100–400 psig (7–28 bar).

10.3.1.4 Gas Purity

Defining what gas purity is needed can be a confusing issue. In general, chromatographers agree that oxygen, water, oils (hydrocarbons), carbon monoxide, carbon dioxide, and halogens in gases supplied to a chromatograph can cause

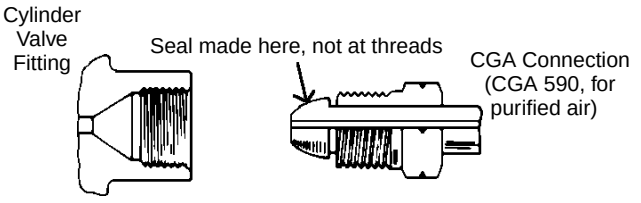


FIGURE 10.5 Sealing point in a CGA connection [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

baseline disturbances, ruin columns, and/or damage detectors (16,17). Gases that meet the specifications in Table 10.5 would meet the needs for all gas chromatography applications. Beyond these points, however, agreement ends. Table 10.6 lists specifications for the gases typically used for general chromatography applications. These are minimum specifications. Some high-sensitivity applications or columns very sensitive to oxidation breakdown require gases of higher purity. Others use only ultra-high-purity gases. Others, with equal experience, use lower grades of gas and depend on inline purification to provide the purity indicated in Table 10.6. Still others argue that there is no need for gas purification and use low grades of gases routinely.

Several facts can bring a rationale to the gas purity arguments. Water and oxygen damage a column by reacting with the phase. Although the exact temperatures at which this damage begins to occur is not known, and probably differs among types of phases and columns, reported damage consistently has been at temperatures of 140°C or above. The injection of air onto columns sensitive to oxygen can show immediate baseline disturbances and long-term column degradation (see Figure 10.6). Similarly, consistently reported are the reaction of oxygen, CO, and CO_2 on detector sensitivity resulting in baseline disturbances and with some special-purpose detectors permanent damage to the detector and

TABLE 10.5 Acceptable Purity Levels for Chromatographic Grade Gases[a]

Gas	Impurity/Maximum Concentration				Total Hydrocarbons
	O_2	H_2O	CO_2	CO	
Helium	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm
Nitrogen	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm
Air	20–22%	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm
Hydrogen	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm
Argon/methane	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm	<1.0 ppm

[a] These limits are set to protect the column. Detector limits usually are less demanding.

TABLE 10.6 Minimum Purity Levels for Chromatography-Grade Gases

Gases for Column and Makeup Uses	
Argon/methane	99.9995%
Helium	99.9995%
Hydrogen	99.9995%
Nitrogen	99.9995%
Gases for Detector Support	
Air (dry)	Zero grade or better
Hydrogen	99.9995%

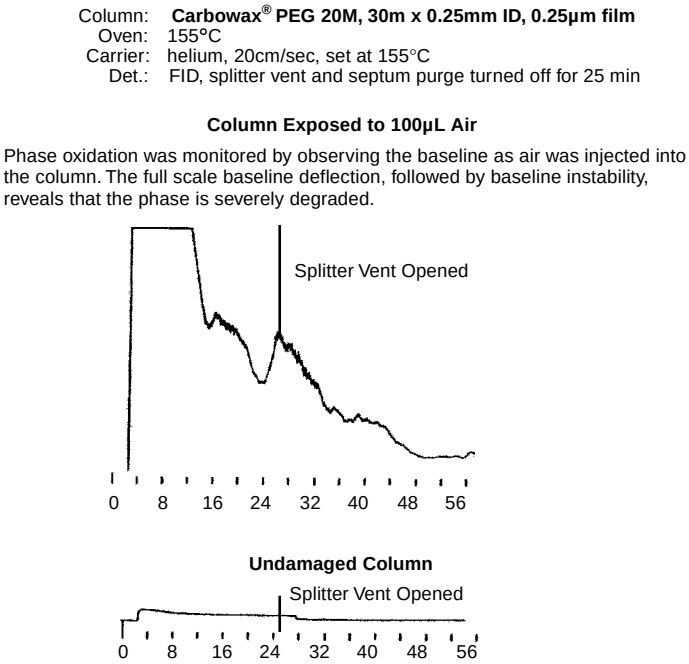

Column: Carbowax® PEG 20M, 30m x 0.25mm ID, 0.25µm film
Oven: 155°C
Carrier: helium, 20cm/sec, set at 155°C
Det.: FID, splitter vent and septum purge turned off for 25 min
Column Exposed to 100µL Air
Phase oxidation was monitored by observing the baseline as air was injected into the column. The full scale baseline deflection, followed by baseline instability, reveals that the phase is severely degraded.
Splitter Vent Opened
0 8 16 24 32 40 48 56
Undamaged Column
Splitter Vent Opened
0 8 16 24 32 40 48 56

FIGURE 10.6 Oxygen degrades thin phase capillary column [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

its sensitivity (see Figure 10.7). The chromatograms in Figure 10.8 show the effect of air, caused by leaks, on polar liquid phases. The reduction of separation is caused by band spreading that is directly related to breakdown of the phase in the column. If you intend to use low temperatures and nondemanding detector sensitivities, you might be able to use gases that do not meet the purity criteria in Table 10.6.

For all except a few selective detectors, however, you should still be concerned about hydrocarbons in your gas, and use gas that has very low hydrocarbon levels. The FID baseline seen in Figure 10.9 is the result of various hydrocarbon impurities found in air. The quality of the air used is dependent often on the source of the air. House air often has hydrocarbons present. Little to no effort is made to design house air systems that will provide hydrocarbon concentrations below 100 ppm. Depending on the grade of cylinder air, you can see some presence of hydrocarbons. The best choice is the use of an electronic air purifier. Although some suppliers of chromatographic products are not concerned about hydrocarbons in gas streams, we strongly recommend using a hydrocarbon trap followed by zero-air electronic air purifier, even if the gas is low in total hydrocarbons.

Not all commercially available regulators are suitable for use with gas chromatographic carrier gases. The critical component is the diaphragm. The Buna-N or neoprene diaphragms in most regulators offgas contaminants, are permeable

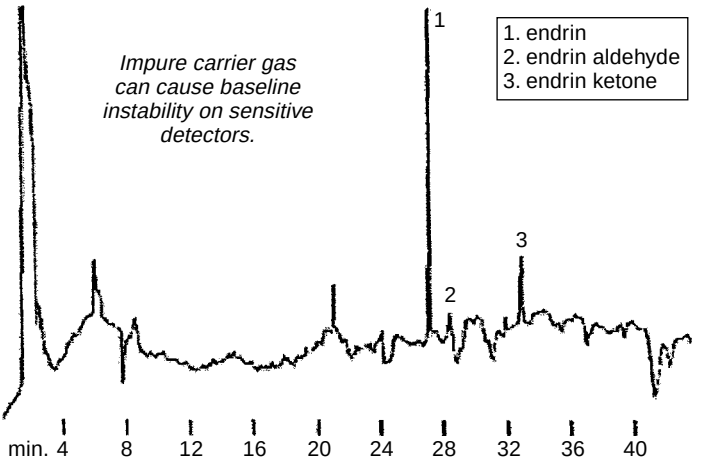


FIGURE 10.7 Impure carrier gas can cause baseline instability on sensitive detectors.

to water, and oxygen (see Scott Specialty Gases laboratory report E-R83-1, request from Scott Specialty Gases, Plumsteadville, PA, USA). A regulator with a stainless-steel diaphragm eliminates these problems. On the other hand, regulators constructed entirely of stainless steel, intended for use with corrosive gases, are very expensive, and are not needed for gas chromatographic applications.

Gas Purifiers Much like the debate over the correct purity for chromatographic gases, analysts have debated the use of inline gas purifiers versus ultra-high-purity gases. Because there are many sources of contaminants in addition to the gas cylinder, we recommend using gas purifiers to protect your instruments. Often, the greatest sources of contaminants are introduced during the process of changing cylinders, which creates an opportunity for room air to enter both line and cylinder. Inline purifiers remove this surge of impurities and keep them from entering the instrument. The second source of contaminants is the diaphragm in the regulator. Most butyl rubber diaphragms will emit hydrocarbons into the gas stream. It is best to use regulators with stainless-steel diaphragms with all carrier-gas lines. Unclean tubing can be a major source of oils and other contaminants (see Section 10.3.2.2). Regulator diaphragms can be a source of hydrocarbons, and oxygen can permeate through the diaphragm. Greases and/or lubricants used in the body of a valve can be sources of hydrocarbons. Every fitting in the system potentially can allow room air, and its associated contaminants to leak into the system (see Section 10.4.1.1). A leak-free system can develop leaks over time, due to expansion and contraction of tubing and fittings with the changing temperatures in the lab. Indicating (color changing) inline purifiers, available for oxygen, water, hydrocarbons, and other contaminants, give visual warning that contamination is present. With some purifiers as the purifier is depleted and reaches its useful life a pressure drop develops between the inlet and outlet and

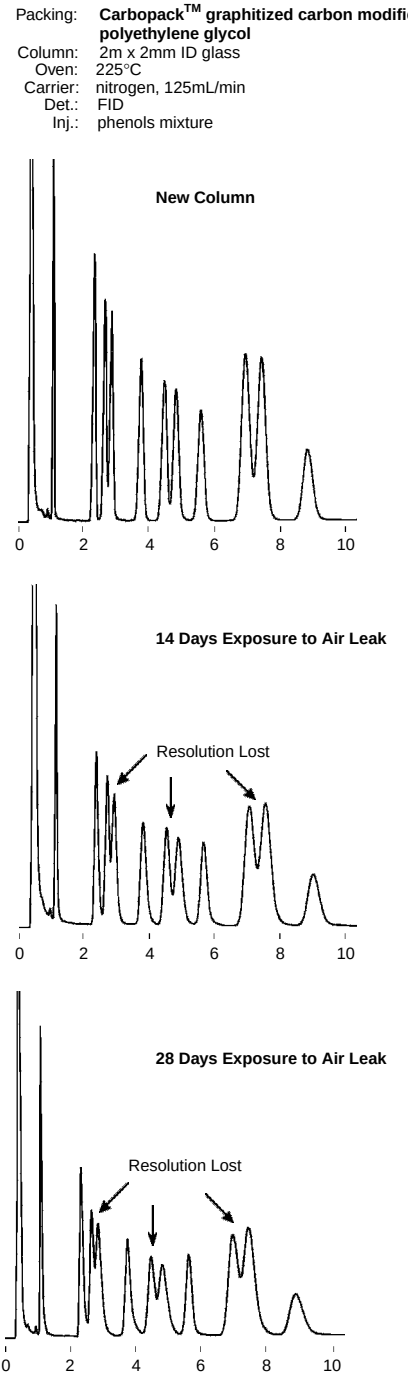

Packing: Carbopack™ graphitized carbon modified with polyethylene glycol
Column: 2m x 2mm ID glass
Oven: 225°C
Carrier: nitrogen, 125mL/min
Det.: FID
Inj.: phenols mixture
New Column
0 2 4 6 8 10
14 Days Exposure to Air Leak
Resolution Lost
0 2 4 6 8 10
28 Days Exposure to Air Leak
Resolution Lost
0 2 4 6 8 10

FIGURE 10.8 Rapid degradation of adsorption chromatography columns [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

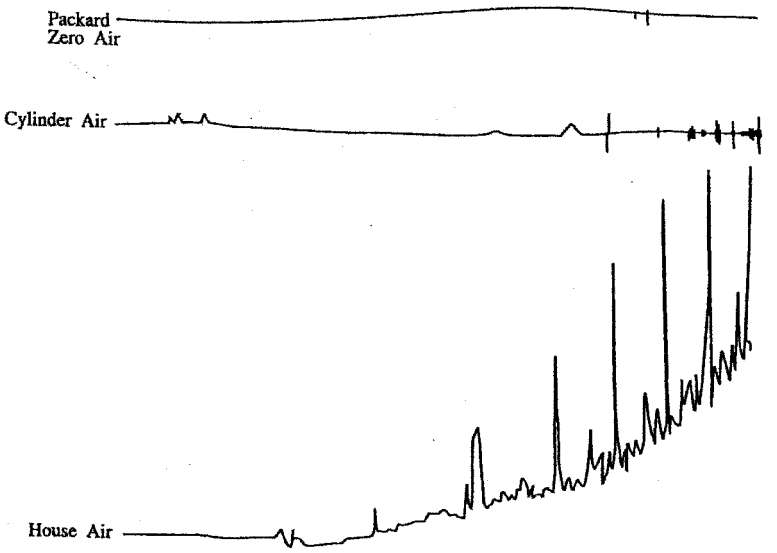


FIGURE 10.9 Baseline disturbance caused by hydrocarbon impurities [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

acts as the signal for changing the purifier. This means installing pressure gauges at each end of the purifier and routinely monitoring the pressure. Change the purifier when the pressure drop reaches 10–15 psig (0.7–1.1 bar).

For highly demanding applications requiring the highest possible gas purity, there are special purifiers and connectors that reduce contaminants to the sub-parts-per-billion level. A combination of special design factors allows a purifier to reach these levels. The catalyst, a nickel material, reacts with a variety of contaminating materials and permanently removes them. The fittings provide better seals due to specially electropolished inner surfaces and special end-fitting designs—faceseal fittings (Figure 10.10). Standard compression fittings are very good, but cannot eliminate trace leaks that allow ppb levels of contaminants. However, few need this challenging level of gas purity.

Carrier-gas purification should start with large-capacity (bulk) purifiers in the order of hydrocarbons, water, and oxygen. Reversing the order of these purifiers shortens their useful life. Smaller-capacity purifiers should be installed in each carrier gas and makeup line and as close to the gas chromatograph as possible (see Table 10.7). The OMI purifier (Figure 10.11) provides final purification of carrier or makeup gas. Its capacity is smaller than that of bulk purifiers but it will provide many months of operation if lines are leaktight and properly maintained. The color indicator in an OMI changes from black to brown showing the degree of use. We also recommend using a hydrocarbon trap in the fuel-gas line.

Additional Comments on Gas Purifiers The color change line visible through the wall of a color-indicating purifier is not a true indicator of the point that

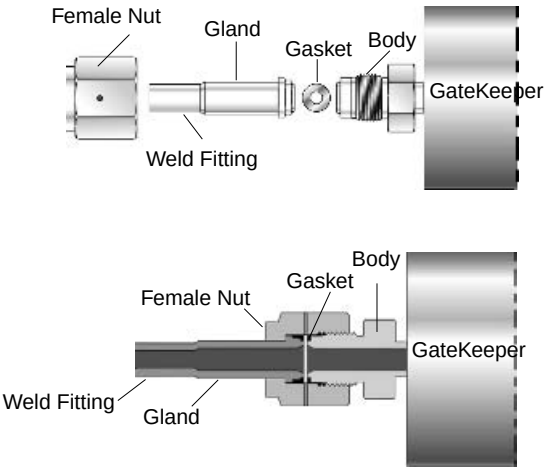


FIGURE 10.10 Faceseal fitting [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

TABLE 10.7 Recommended Inline Gas Purifiers

Purifier	Removes	From	Indicating
Supelpure-HC Trap	Hydrocarbons	All gases	No
Molecular sieve 5A	Water, heavy hydrocarbons	Air, hydrogen, nitrogen, helium	No
High-capacity purifier (heated purifier)	Oxygen, water	Helium, nitrogen *Do not use with hydrogen or air*	Yes (*pressure*)
OMI	Oxygen, water, CO, CO_2, alcohols/phenols, sulfur- and halogen-containing compounds	Argon, helium, nitrogen, hydrogen, argon/methane, neon	Yes (*color*)

the purifier material is expended. There is a tunneling or funneling aspect to the purification process—the core of the purifier is expended before the outside edges. Therefore, you should change the purifier when the color change is about 75% along the tube—the front of the cone of expended material will be much closer to the outlet end of the tube.

Not all purifiers on the market are adequate for use in carrier-gas lines and for this reason columns often fatigue before a color change is visible. Purifiers constructed from plastic tubing tend to allow water and oxygen to permeate into the gas line (18). Most water-removing purifiers using Drierite and similar color indicators do not completely remove water. Also avoid purifiers that have O-ring

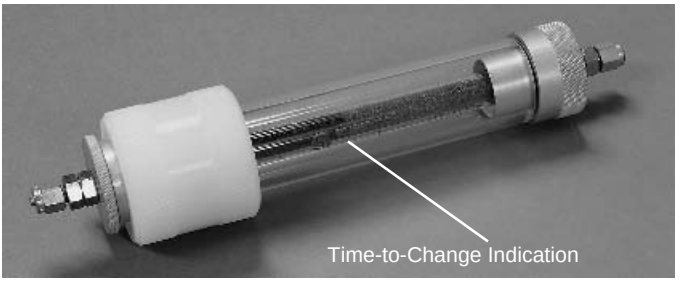


FIGURE 10.11 Partially used OMI purifier with "time to change" marking [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

seals; they typically leak, especially on retightening or on the second installation. Purifiers that are constructed of plastic, and filled with low-efficiency adsorbents, are adequate for air lines.

Inline Filters To protect needle valves, regulators, flow controllers, and other devices, each gas line should contain a filter capable of removing particles 7–10 μm in diameter. The filter in a two-stage regulator will not trap particles this fine.

10.3.1.5 Regulators and Associated Connectors

A well-designed gas supply system uses several types of regulators. Each gas cylinder is equipped with a two-stage regulator. With a two-stage regulator the first stage reduces the pressure of gas coming from the cylinder to 300–500 psig (21–35 bar), and then the second stage reduces the pressure to the pressure desired in the mainline (Figure 10.12). You control the pressure for the second

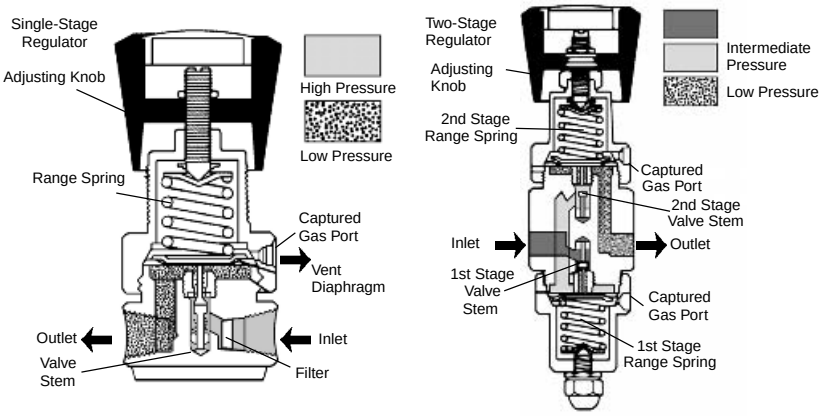


FIGURE 10.12 Gas flow paths in single-stage and two-stage regulators [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

stage; the first stage pressure is preset by the regulator manufacturer. Do not use single-stage regulators on gas cylinders. With a single-stage regulator you would have to adjust the mainline pressure as the pressure in the cylinder fell off. With a single-stage regulator, downstream line pressure will increase at a rate of 0.65 psig per 100 psig (0.05 bar per 7 bar) decrease in cylinder pressure. This change in downstream pressure may be unacceptable. (*Note:* Two pressure gauges do not always denote a two-stage regulator. Some single-stage regulators have an inlet gauge and an outlet gauge.)

In multiple-unit gas chromatographic systems, the branchline to each chromatograph should include a single-stage regulator, to step down the pressure in the line to that required by the instrument. There is another reason why both two-stage and single-stage regulators are used in a multiunit gas chromatographic system. To ensure effective operation, you must maintain at least a 10−15-psig (0.7−1.1-bar) pressure differential across all flow- and pressure-controlling devices (Figure 10.13). Pressure in the mainline can change because of new demands, because the cylinder pressure output is not properly reset when a cylinder is changed, because the system has long plumbing lines (pressure will be lowest at the most remote instruments), or because pressure will vary during a temperature-programmed analysis. To ensure that you maintain a 10−15-psig (0.7−1.1-bar) pressure differential, you must know the pressure of the gas as it enters the gas chromatograph and at the head pressure gauge on the instrument. A system with a two-stage regulator at the cylinder and a single-stage inline regulator at each chromatograph provides this information. Without a single-stage regulator just before each instrument, changes in the main-line pressure will affect the operation of the individual gas chromatographs.

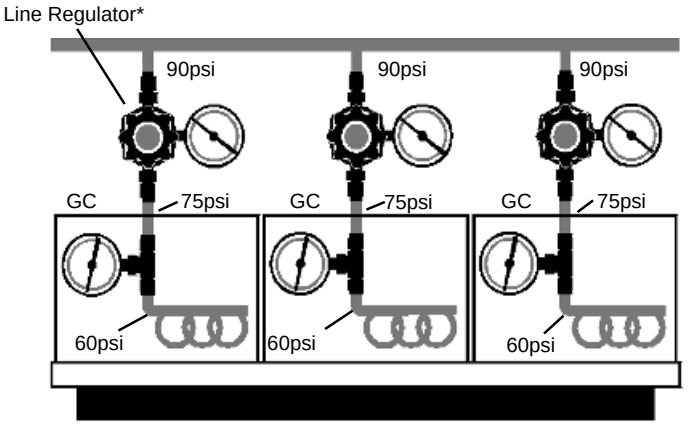


FIGURE 10.13 To ensure effective operation, maintain at least a 10−15-psig pressure differential across all flow and pressure-controlling devices [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

Gas generators develop gas pressures much lower than the pressures delivered from cylinders. A single stage regulator is suitable for regulating gas flows from these devices.

Regulators used for air can be fitted with a neoprene diaphragm. These regulators will reduce installation costs without sacrificing the integrity of the system.

Additional Comments on Regulators Modern gas chromatographs are factory-set to operate at column head pressures of up to approximately 60 psig (4 bar). Very long columns (e.g., ≥100-m capillary columns) can require column head pressures up to 90 psig (6.3 bar) or higher. Typically, the line pressure should be 15–20 psig (1.1–1.4 bar) higher than the inlet pressure to the gas chromatograph. As discussed above, for pressure regulators and flow controllers to work correctly, there must be a 10–15-psig (0.7–1.1-bar) difference between the input pressure and the maximum output pressure. Consider these values in deciding what pressures to use in your system—higher pressures may require you to change some internal pressure gauges, flow controllers, or pressure regulators in your gas chromatograph.

Never remove a two-stage regulator from a gas line with a high pressure isolated in the first stage—the sudden release of pressure could rupture the diaphragm, ruin diaphragms in downstream regulators, and/or create gaps in a packed column (the packing could even be forced out of the column). Always depressurize a two-stage regulator through the second stage (or allow gas to completely bleed out of system, i.e., a second-stage regulator gauge will read zero). If your system has a single-cylinder gas supply, or a gas generator, the first step is to turn off the gas chromatograph oven and let the column cool. In a two-cylinder system, transfer flow to the second cylinder. Next, close the first-stage (cylinder-side) valve on the regulator to be removed from service, while leaving open the shutoff valve after the regulator. This will allow the gas remaining in the regulator to pass through the regulator. Vent the pressure through the system (be sure that the column is cold), through a vent installed in the gas line, or through the vent on the regulator itself (some models). Finally, close the downstream pressure control valve and remove the regulator.

Whenever you change cylinders or regulators, be sure to protect the columns in the chromatograph. Before you disrupt the gas flow, either switch to a second source of gas or, if you are disrupting carrier-gas flow, turn off the oven and cool the column supplied by the gas.

Always use a regulator rated for your intended application. Never switch CGA or other fittings to use a regulator for a purpose for which it was not intended (e.g., do not refit any regulator for oxygen delivery). Table 10.8 lists the proper CGA fittings for each type of gas used for gas chromatograph. Never switch gauges or inlet fittings and never change gas service. Never close a regulator body in a vise to remove a fitting—this almost certainly will break the diaphragm seal and cause the diaphragm to leak. Never lubricate a regulator or use pipe sealant.

The regulators you should use would have a maximum of 100 psig (7 bar) output pressure and gauges that read to 200 psig (14 bar) in 5-psig (0.4-bar)

TABLE 10.8 Regulator Fittings

	CGA Connections (All 0.965 in. or 2.45 cm)		
Gas	Outlet Description[a]	Nipple Shape	Nut
Air (purified)	590-14LH	Bullet	Male
Argon	580-14RH	Bullet	Male
Argon/methane	350-14LH	Round	Female
Helium	580-14RH	Bullet	Male
Hydrogen	350-14LH	Round	Female
Nitrogen	580-14RH	Bullet	Male

	Other Connections		
Gas	DIN 477	BS 341	Japan
Air (purified)	13	3	—
Argon	6	3	W22-14RH
Argon/methane	1	4	—
Helium	6	3	W20.9-14LH
Hydrogen	1	4	W22-14LH
Methanol	1	—	—
Nitrogen	6	3	W22-14RH

[a]LH—left-hand; RH—right-hand. There is always a groove in a nut with left-handed threads (see Figure 10.3).

intervals. Regulators that can provide much higher pressures are available, but we do not recommend these for gas chromatograph use. It is difficult to regulate a pressure to within a few pounds per square inch gauge or bars on a 2000-psig (140-bar) regulator with gauge gradations in 20-psig (1-bar) intervals.

Be sure to indicate, on or near each regulator in your system, the pressure that the regulator should be reset to after a cylinder change—you might not always be present when a cylinder is emptied and replaced.

The American Society for Testing and Materials (ASTM) summary book on chromatography, and related ASTM publications, provide "lab-tested" guidance to the practicing chromatographer (order from American Society for Testing and Materials, 100 Bar Harbor Drive, West Conshohocken, PA 19428-2959, USA).

10.3.2 Tubing and Plumbing

10.3.2.1 Tubing Choices

Many types of tubing are available for supplying air, helium, nitrogen, argon, and gas mixtures for gas chromatography. In practice, however, only copper and stainless steel are viable alternatives. Table 10.9 lists the specifications for tubing suitable for use in gas chromatography. Before use, this tubing must be cleaned to remove traces of oil and dirt. Most tubing labeled as cleaned, and offered in chromatography catalogs, is adequate for plumbing gas lines.

TABLE 10.9 Tubing and Tubing Preparation for Gas Chromatography

Tubing Type	For Columns	For Plumbing
Stainless steel	Premium grade 304	Regular grade 304
Copper	Highly cleaned[a]	Cleaned
Aluminum	Highly cleaned[a]	Not recommended
Nickel	SP-Alloy (T-1)	Not recommended
Teflon	TPE or FEP	Not recommended
Tygon	Not recommended	Air lines only

[a] Not recommended for columns today.

Column-quality (chromatographic grade) tubing receives acid and base cleaning, and is tested for active sites. This extra treatment is not needed for gas lines. Tubing obtained from building supply houses or hardware stores is not clean enough for use—we have seen oil dripping from the vent ports of TCD detectors when non-chromatographic-grade tubing was used, uncleaned, to plumb a gas chromatographic system. Similarly, the dirt in commercial tubing can clog the frits in flow controllers and other fine metering valves, and ruin these devices. To be sure of the quality of the tubing used in your lab, discuss Section 10.3.2 of this chapter with the suppliers and fabricators of your gas supply system. See Section 10.3.2.2 on how to clean tubing.

Stainless Steel Tubing Strong and reusable, stainless-steel tubing is always the best choice—and the most costly—for a gas chromatographic system. For hydrogen, mill-finished, oxygen-cleaned 304- or 316-grade stainless-steel tubing (never copper) should always be used. Care should still be taken to clean this material. For special applications where pristine conditions are needed, such as with helium ionization detectors, 304 L electropolished (EP) stainless steel with vacuum-coupled replaceable (VCR) connections and orbital weld joints is the best choice (available through GOW-MAC and Antek Instruments). An electropolished surface significantly reduces water and contaminant capture. It can be very expensive, but for critical applications, it is worth the added expense.

Copper Tubing Because of its lower cost, copper tubing is the most commonly used plumbing material in gas chromatographic systems. Copper should not be used with hydrogen gas, nor where the gas line might be flexed. With time copper tubing of any diameter work-hardens and is very easily broken during flexing. Only very short lengths of $\frac{1}{8}$-in. (3.18-mm)-o.d. copper tubing should be used because copper tubing has a much smaller inside diameter than does stainless-steel tubing (1.65 vs. 2.1 mm). Long lengths lead to high backpressures (even stainless-steel gas lines should be as short as possible). By far the most common diameter of copper tubing used is $\frac{1}{4}$-in. (6.35-mm)-o.d. tubing, as it tends to be stronger than $\frac{1}{8}$-in. (3.18-mm)-o.d. tubing, but with flexing it can still break; $\frac{1}{2}$-in. (12.7-mm)-o.d. copper tubing typically is inflexible. You will need Swagelok or

TABLE 10.10 Recommended Lengths of Connection Tubing to Gas Chromatographs

Material	Outside Diameter	Maximum Length
Copper[a]	$\frac{1}{8}$ in. (3.18 mm)[b]	6 ft (1.8 m)
	$\frac{1}{4}$ in. (6.35 mm)	20 ft (6.1 m)
	$\frac{1}{2}$ in. (12.7 mm)	100 ft (30.5 m)
Stainless steel	$\frac{1}{16}$ in. (1.59 mm)	2 ft (0.6 m)
	$\frac{1}{8}$ in. (3.18 mm)	10 ft (3 m)
	$\frac{1}{4}$ in. (6.35 mm)	20 ft (6.1 m)
	$\frac{1}{2}$ in. (12.7 mm)	100 ft (30.5 m)

[a] Do not use with hydrogen.
[b] Not recommended due to brittleness.

soldered fittings for all direction changes and connections in $\frac{1}{2}$-in. (12.7-mm)-o.d. copper lines (see Table 10.10).

Additional Comments on Tubing Never use cast iron or black steel pipe to supply gases to chromatographs. Over time, these materials will form rust that will travel through your system, ruining valves, regulators, and other components.

Soft and easily kinked when new, aluminum tubing, like copper tubing, becomes brittle over time, forming aluminum oxides. Because aluminum tubing offers no particular advantages relative to copper, and has a higher degree of the same disadvantages, we do not recommend using aluminum tubing to plumb a gas chromatographic system.

Teflon and nylon tubing are acceptable for air and actuation lines, but permeability to water and oxygen precludes the use of these or other polymeric materials for most gas chromatograph plumbing needs, including carrier-gas and makeup-gas lines. Hydrocarbons from some polymeric tubing can appear as impurities in the system. Even in a well-designed plumbing system, a regulator can fail and release full cylinder pressure into a line, and the line will withstand the pressure. Polymer tubing will not pass this test.

10.3.2.2 Cleaning

Dirt and oil are present in all tubing because of the manufacturing process. Only if you buy cleaned tubing from a chromatography supplies dealer will you receive tubing immediately ready for chromatography. Even then, the tubing must be capped to keep dust and dirt out during shipment and the system assembly process. During cutting and assembly processes, metal fragments and dirt can get into the tubing. It is best to clean the tubing, blow it out, and then purge the tubing.

The first concern is the removal of dirt and oil used during the manufacturing process of the tubing. If one intends to use an electron-capture detector at any

time, do not clean the tubing with chlorinated solvents. Using a nonpolar solvent such as *n*-hexane, flush the tubing until the solvent flowing out of the line is clear. Allow sufficient time for the solvent to dissolve materials in the line. Rinse the tubing with water to flush the hexane and absorb any free acidic or basic material. Next, flush with methanol to remove traces of hydrocarbons and the water remaining in the tubing. Using clean nitrogen (not compressed air, which always contains some oils), attempt to remove all traces of methanol. An additional recommended step involves heating the tubing. The coiled tubing can be put in a large oven, and heated to 110°C, during the nitrogen purge, but only after the bulk of the organic rinsing agents has been purged.

Clean tubing should be capped or sealed in some fashion to keep dirt out. If caps are not available, flatten the ends of the tubing and fold each flattened end back on itself (Figure 10.14).

10.3.2.3 Cutting, Reaming, and Bending

To avoid creating kinks or flat spots as you uncoil the tubing, hold the coil of copper or stainless-steel tubing perpendicular to a table or the floor. Hold the end of the tubing with one hand and roll the coil away from you with your other hand (Figure 10.15).

The preferred tool for cutting copper or stainless steel tubing is a tubing cutter that presses a cutting wheel against the tubing while the device is turned repeatedly around the tubing. This tool makes a very clean, truly perpendicular cut that allows the tubing to fit squarely into a fitting. Some cutting machines also work very well, especially for cutting $\frac{1}{16}$-in. (1.59-mm)-o.d. tubing. In contrast, general-purpose tubing cutters distort the end of the tubing and handheld saws often leave ragged and angled cuts and excessive metal filings in the tubing.

Reaming should always follow cutting. When cutting any tubing, but especially when cutting copper, a soft metal, the metal typically intrudes inward and reduces the inner diameter of the tubing, sometimes almost completely closing it. Special care must be given to reopening the tubing to its original inside diameter. Use

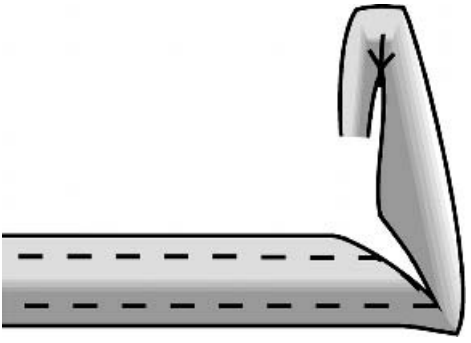


FIGURE 10.14 Tubing crimped and folded to keep dirt out [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

FIGURE 10.15 Uncoil tubing without creating bends [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

a reaming tool to carefully cut away excess metal and slightly bevel the inside edge of the tubing (Figure 10.16). Remember to clean the metal filings out of the tubing, or they will be pushed into the nearest valve, flow controller, or pressure regulator, where they could cause damage. Direct a steam of clean, dry nitrogen gas through the tubing to remove the filings. Do not use air from a compressor; it might contain oils.

Bend tubing very carefully, taking care not to kink tubing, which will reduce the inside diameter or create flat spots. Use tools designed for this purpose. If the tubing at a bend is visibly flat, discard it. If you need a very sharp bend or there is not room for a bend, use an elbow fitting.

Flexible Hoses Most tubing is not designed for continual flexing. This creates a problem for attaching a regulator to a cylinder or to a gas line. The solution is a flexible metal hose: a 30″ (76-cm) length of corrugated (bellows) stainless-steel tubing reinforced with stainless-steel braids, with additional casing on the outside, and fitted with compression fittings, pipe thread (male), or CGA connectors. The hose can be used to connect a cylinder to a fixed, wall-mounted regulator or to connect a regulator mounted on a cylinder to a gas line that is secured to a bench or wall. Use hoses rated to 3000 psig (210 bar) for the gas you will be

FIGURE 10.16 Trim and bevel a newly cut tubing end [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

using. When the regulator is removed from the cylinder, it should be properly supported—do not suspend it by the hose.

10.3.2.4 Valves and Fittings

In addition to the tubing, all other system components (joints, valves, pressure-relief valves, flash arrestor, etc.) must be compatible with anticipated operating pressures and temperatures. Trace contaminants usually come from O-rings, washers, elastomers, and plasticizers sometimes used in valve or other devices. Avoid this problem by eliminating elastomer valve seats and using metal-to-metal seals for all joints and seals (military specification grades of Teflon, T-27730A are acceptable and provided by most chromatography supply houses). Greased fittings and soldered lead joints should not be used because of potential contamination from organic greases or acid solder flux. When using copper tubing, compression fittings or well-fabricated brazed joints usually provide leaktight connections, and $\frac{1}{4}$-turn or $\frac{1}{2}$-turn bellows or diaphragm-type valves assure the best positive shutoff of gas flow.

Pressure Gauges Pressure gauges should be selected by pressure delivery range that should slightly exceed the pressure you anticipate using. It is very difficult to read 10- or 20-psig (0.7- or 1.4-bar) increments on a 2000-psig (140-bar) gauge. Most gas chromatographic systems will not need gas pressures exceeding 100 psig (7 bar). Pressure gauges have pipe threads and they should be attached using Teflon tape. Never use pipe sealant.

Pressure-Relief Devices A pressure-relief device is required with any flammable gas (e.g., hydrogen), whether delivered from a generator or a cylinder. A hydrogen pressure-relief valve, is different from most pressure-relief valves in that it is designed to accept fittings that allow additional plumbing and proper venting. Most hydrogen generators will have an internal device. The outlet of the internal relief valve should be properly plumbed to a safe vent. If you cannot confirm that your generator has such a device, install one downstream of the generator, in conjunction with a flash arrestor (Figure 10.17).

The pressure-relief device in a hydrogen line should always be safely vented. Mixtures equal to or greater than 4% or more hydrogen in air are explosive. Do not allow these concentrations to form in the lab. Vent hydrogen to a fume hood or other conduit leading out of the building. Check with your safety department to determine the proper venting procedure for your site.

We recommend that you install a pressure-relief valve that releases pressures above 2000 psig (140 bar) on each main gas line, to protect downstream equipment from high-pressure failures. The best location for the device is after the regulator shutoff valve (Figure 10.17). Pressure-relief valves on nonflammable gas lines need not be vented to a hood, but be sure to direct the vents downward (away from operator).

Dry Flashback Arrestor In the event of a hydrogen flashback, a dry flashback arrestor diverts the flame into a 3 ft (1 m) length of tubing, where the

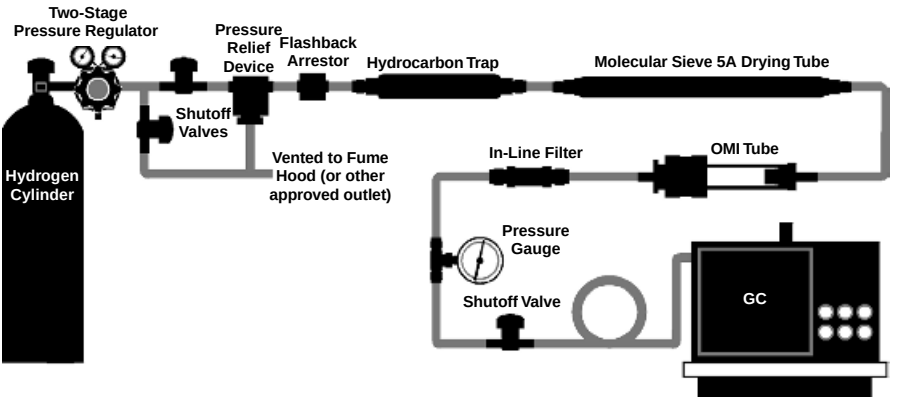


FIGURE 10.17 Safely designed hydrogen line (carrier gas) system assembly [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

flame is extinguished and the heat is absorbed. The shockwave preceding the flashback closes and locks the arrestor's shutoff valve, eliminating continued gas feed. Install the dry flashback arrestor after the shutoff valve and pressure-relief device for the cylinder regulator (Figure 10.17). Many hydrogen generators incorporate a flashback arrestor. If your generator does not, install one downstream of the generator (available at most chromatographic supply houses). Use only devices that meet Occupational Health and Safety Administration (OSHA) and National Fire Protection Agency (NFPA) codes, or overseas equivalents, and are Factory Mutual–approved. Devices larger than the one shown on these are available commercially. Dry flashback arrestors are reusable and can be reset but be sure to determine and eliminate the cause of the flashback before resetting the arrestor.

In contrast, wet flashback arrestors, which incorporate ethylene glycol, should not be used with chromatographic systems. Although ethylene glycol is only weakly volatile, ethylene glycol vapor could be released into the gas system. This contamination will cause unstable baselines and high background signals.

10.3.2.5 Making Connections

Installation of all the lines, regulators, valves, and other associated hardware needed in a gas chromatographic system requires an assortment of tube, threaded pipe, and, perhaps, soldered connections. When tube connections are required, always use highest-quality fittings. Only threaded pipe connections external to the gas chromatograph should be sealed with instrument-grade Teflon tape. Pipe sealant (pipe dope) or other chemicals, and some lower-grade Teflon tapes, contain organics that could bleed into the gas stream and should be avoided. Roll a layer of the tape onto the threads counter to the direction of the threading (i.e., counterclockwise) and tighten the tape. Thread the two parts together and tighten.

When soldered connections are needed, the brazing alloy should be flat stick silver solder containing 15% silver. Use MAPP (methylacetylene propadiene),

rather than acetylene, when soldering with this high-melting-point solder. *Use no flux*. Flux will cause interference with electron capture detectors, and possibly with other detectors and some columns.

Assembling a Compression Tube Fitting Before assembling a nut and ferrule on the metal tubing, inspect the tubing to be sure, the surface is smooth and free of longitudinal scratches, and the cut end is deburred. If the tubing is acceptable then slide on the nut with the open side facing the end of the tubing. Next, slide on the back ferrule with the wider part facing the nut. Then slide on the front ferrule with the small end of the cone facing the end of the tubing (Figure 10.18). Push the assembly about 1 in. (2–3 cm) onto the tubing. The ferrules and nut should slide onto the tubing easily and rotate freely. Insert the tubing into the fitting—it should fit easily. *Hand-tighten* the nut/ferrule assembly onto the fitting. Then, using two wrenches, tighten the assembly. We do not recommend relying on torque measurements, due to differences in tubing wall thickness and materials of construction. Use only brass ferrules with copper tubing and stainless-steel ferrules with stainless-steel tubing.

Instead, monitor the number of turns you make on the nut. If the parts are clean and properly assembled, $\frac{3}{4}$-turn on $\frac{1}{16}$-in. (1.59-mm) or $\frac{1}{8}$-in. (3.18-mm)-o.d. tubing, or $1\frac{1}{4}$ turns on $\frac{1}{4}$-in. (6.35-mm)-o.d. tubing, should seal the fitting. A properly tightened compression ferrule system resembles Figure 10.19 (M–1). Notice that a properly seated front ferrule will be forced slightly into the tubing. Always use two wrenches when tightening fittings, one to hold the fitting in place and the other to tighten the nut–ferrule assembly. A tee wrench is very useful for tightening tees. A hydraulic swaging unit might be required to swage $\frac{1}{2}$-in. (1.27-mm)-o.d. or larger fittings. If the fitting does not seal properly, additional tightening seldom provides a leak-free seal. Disconnect the nut and examine the inner surfaces of the fitting, ferrules, and tubing for dirt or scratches. If necessary, replace defective components. Figure 10.19 (M–2) shows the effects of overtightening a fitting. Notice the concave front ferrule. The shoulder on the distorted ferrule is typical of overtightened ferrules. The cross-sectional profile of a good ferrule is a straight edge from the tubing to the back edge of the ferrule. Never use sealing compounds on the outside of fittings to stop leaks.

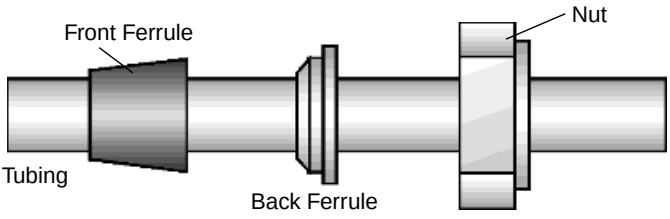


FIGURE 10.18 Assembling a compression fitting [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

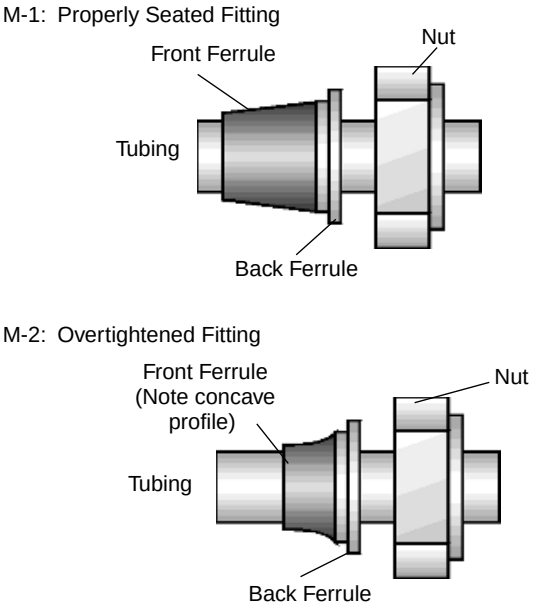


FIGURE 10.19 Ferrule profile reveals correct/incorrect tightening [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

There should be no need to disassemble and inspect a compression fitting if it passes your leak-testing procedure, but in some facilities additional testing is required to ensure that a fitting has been sufficiently tightened. The preferred test involves using gap inspection gauges. A gap inspection gauge has a thick end and a thin end. Attempt to insert the thick end of the gauge in the gap between the nut and body of the tightened tube fitting. If the thick end will fit, the fitting nut has not been adequately tightened. If the thick end will not fit, the minimum requirement for tightening has been met.

Properly installed compression fittings can be disconnected and reconnected many times. To reconnect a fitting, simply hand-tighten the nut, then slightly tighten it with a wrench. It should take little additional pressure to tighten the fitting, because you are simply making a metal-to-metal seal between the ferrules and the body of the fitting, not reseating the ferrules onto the tubing. Always confirm that the reconnection is leak-free, using an electronic leak-detecting device.

Additional Comments on Connections Never mix tube-fitting components of different brands. Although products from different manufacturers appear to be interchangeable, they are not. Nuts, ferrules, and bodies will have different angles and depth specifications. Two-piece ferrules and one-piece ferrules have different mechanical sealing functions between the tubing and the fitting body. Decide on a fitting manufacturer and stay with the decision throughout your plumbing system.

10.4 SYSTEM INSTALLATION AND ASSEMBLY

10.4.1 System Assembly

Your plumbing assembly should follow the single-gas chromatograph, 1–4-gas chromatograph, or 5–20-gas chromatograph system described later in this chapter. Figures in these sections show our recommendations for the various types of valves, regulators, and other devices for each system. We also recommend that you read the Swagelok *Tube Fitter's Manual*, especially Chapter 3, *Tubing and Tube Fitting Handling and Installation*. The manual offers many tips and helpful directions that go beyond the detail in this chapter.

Securing Fittings and Tubing Secure valves and gas-supporting lines to benches or walls to avoid flexing the tubing when opening or closing a valve. Many types of fasteners are available for tubing, and brackets are available for most valves. Fasten down tubing every 4–6 ft (1.8 m).

Although most of the plumbing in your system should be secured to a bench or wall, there should be some flexibility at the point of connection to the gas chromatograph. It is a good idea to roll about 3 ft (0.91 m) of the gas line between the shutoff valve and gas chromatograph into a coil 4–6 in. (10–15 cm) in diameter (Figure 10.20). This will allow some lateral and front-to-back movement of the instrument when it is being serviced.

Plumbing Two Gases Together Some analysts frequently switch carrier gases as the applications for an instrument change. Never attempt to plumb two carrier gases through the same line, through tees, valves, or other arrangements. Even with shutoff valves, flowcheck valves, and other devices that are supposed to

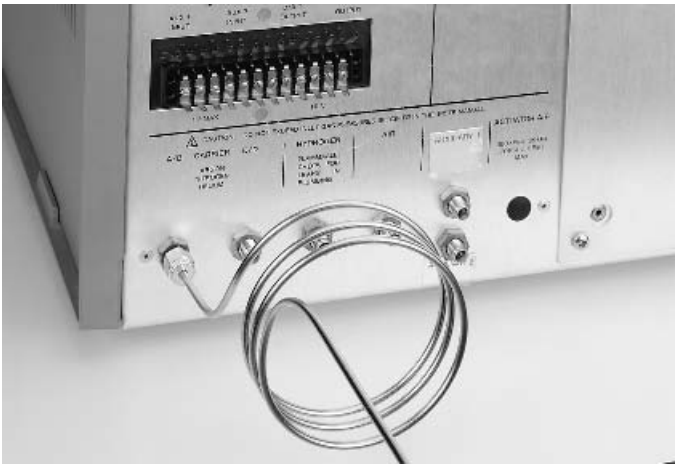


FIGURE 10.20 Coiled tubing allows flexibility in installation of a gas chromatograph [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

guarantee that the two gases never mix, in time they do mix, usually through human error. It takes little time to disconnect one line and attach another, and this is the best approach. If time is critical, you can use quick connect fittings for this purpose. If you use quick-connect fittings, however, we strongly suggest that you routinely test for leaks, and include oxygen and water vapor traps downstream.

Coding Gas Lines In multiunit installations it is important to know what gas a valve will deliver when you open it, but when all the gas lines are in place it can be difficult to discern what line contains what gas. Color-code (i.e., paint) or label each line so that analysts and repair technicians can quickly determine what gas each line contains. You can buy colored sleeves, tags, or other types of labels, or simply paint the lines in different colors.

10.4.1.1 Finding and Eliminating Leaks

Equipment Alternatives Testing a system for leaks is often considered very difficult. In truth, the initial pressure test of the entire system is very simple. If the system passes this initial test, it is ready to use. Finding the leaks, if they exist, can be more difficult. Often a cylinder of oil-free air is used to first check each of the gas lines in a new system for leaks—it is costly to use high purity gases, and it is not safe to leak test with hydrogen. On the other hand, helium or nitrogen will allow leak testing if part of the system fails the pressure test. We recommend using high-purity helium, or a lower grade of helium passed through traps that will remove hydrocarbons, oxygen, water, and particles (see Section 10.4.2). Helium is the easiest gas to detect with an electronic leak detector.

Pressure testing reveals the presence of leaks, but does not show where they are located. You must find leaks by using either a liquid (e.g., Snoop) or an electronic leak detector. *We recommend that all leak testing be done with an electronic leak-detecting device, not with liquids of any kind.* Just like a laboratory sink aspirator will draw a vacuum on a small side line while water is running through the mainline, a leak—small or large—will draw in gas or liquids as it allows gas to leak out. If there is a leak in a line, any liquid leak detector could be siphoned into your system and could reduce sensitivity or cause a drifting baseline. To avoid any chance of contamination, we strongly recommend using an electronic leak detector. Use of Snoop or a soap solution can be problematic with a nitrogen–phosphorus detector

Electronic leak detectors are simple to use. Simply set the readout to zero, as only air is being drawn into the unit, then place the probe at the site to be tested and sample the air around the site (Figure 10.21). The detector, a form of TCD, senses the thermal conductivity of the gas in the detector cell. If the detector senses gas mixtures other than normal air, the needle on the gauge will be deflected, indicating a leak. The detector is very sensitive for helium and hydrogen. Although less sensitive for nitrogen (air is 80% nitrogen, so the differences in thermal conductivity are small), its nitrogen-sensing capability is

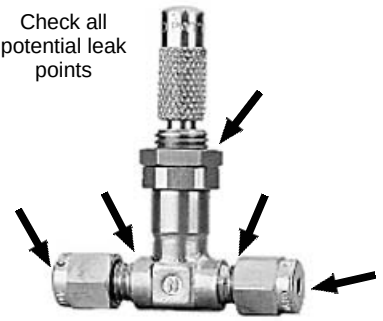


FIGURE 10.21 Leaks checking a valve with an electronic leak detector [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

as good as that of liquid leak detectors. Obviously, you cannot use this device to test air lines for leaks.

Testing for Leaks (*Caution*: Always wear eye protection and gloves when opening or closing cylinders. Do not stand in front of the gauges. Bourdon tubes in pressure gauges can rupture with enough force to cause serious injury. Also, bypass or remove purifiers during leak testing.) Open all valves in the line (and in each branch line in a multiple-gas chromatographic system), but close the last shutoff valve just before the gas chromatograph(s). Pressurize the system to 100 psig (7 bar). After a few minutes, when the pressure is stable, close the shutoff valve immediately downstream from the cylinder regulator. You may see an initial pressure drop of a few pounds (it might be necessary to install a pressure gauge after the shutoff valve for this test). If the system then maintains pressure for $\frac{1}{2}$ hr, you have no leaks of any significance. If the pressure continues to drop, you will need to search for leaks (typically it will drop very rapidly if leaks exist). In a multiple-gas chromatographic system, shutoff valves at each branch and just before each gas chromatograph enable you to isolate and test sections of the system. This is quicker than testing every fitting, regulator, and valve.

If your system fails the pressure test, and you have been pressure testing with air, vent the air in the section(s) involved and repressurize with helium. Using an electronic leak-detecting device, systematically isolate and test each section, starting at one end of the system and working back to the cylinder. If you find a leak, seal it, and then pressure-test again before proceeding. Test *each* line connection, each valve knob connection, the vent holes of regulators, or anywhere you feel a leak could develop. Do not forget to test connections inside the chromatograph.

Most regulators have a small vent hole on the spring side of the diaphragm (Figure 10.22). If gas is leaking from this site, the diaphragm or an internal seal is bad. If the leak is from the pressure-relief port, that part of the regulator may

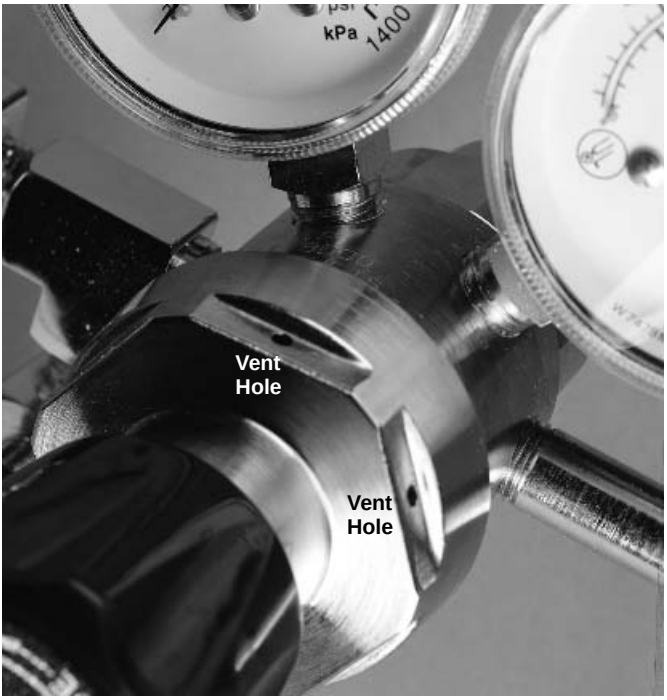


FIGURE 10.22 Be sure to leak-check all regulators [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

be bad. If the leak is internal to the regulator—a leaking diaphragm, internal seal, or pressure-relief vent—replace the regulator. Never attempt to repair or replace any parts other than the CGA fitting. Faulty regulators should be sent to people who are properly trained in regulator maintenance.

If the leak is between the CGA fitting and the regulator the fitting may simply be loose and need tightening. Most CGA fittings have flat surfaces for tightening. Do not overtighten. If the leak persists, unscrew the CGA fitting and ensure that there is no dirt on the fitting or cylinder seat. If dirt is not the problem and the leak persists, you may need a new CGA fitting, or the seat of the fitting in the cylinder valve may be damaged. If the fitting or the seat is damaged, using Teflon tape on the fitting will not work—the sealing point is at the end of the fitting, not on the threads (Figure 10.5). After you find and seal all leaks, it is time to fill the lines with the correct gases.

10.4.1.2 Purging

Once you have determined that the system is leak-free, you are ready to purge the lines and replace the air, nitrogen, or helium test gas in each line with the gas for which the line is intended. The procedure to follow depends on the gas line you are purging. For air and hydrogen as detector gases, use the simple purging

procedure for fuel gases described below. Carrier-gas lines and makeup-gas lines require a more extensive purge to ensure the desired purity level.

(*Note*: In each case, purge the line up to the first purifier, bypass the purifiers, or remove the purifiers during purging. Do not purge the line through the purifiers or the chromatograph.)

Simple Purging Procedure for Fuel Gases (*Caution:* Be sure to properly vent hydrogen during this stage. Trained personnel should be present, testing with a portable low-explosive-level meter, to ensure that you do not create explosive concentrations of hydrogen.) Open all valves in the main and branch gas lines. Slowly open the valve on the cylinder, pressurizing the two-stage regulator. Slowly open the downstream pressure control knob on the regulator, to allow gas to flow through the lines at a pressure of 5–10 psig (0.4–0.7 bar). Purge the lines for 5 min, and then close the shutoff valves at the vent ends of the lines. This may involve several points if you have a manifold system with several branches—start at the branch closest to the cylinder and work out. Now turn off the cylinder and isolate it by closing the shutoff valve downstream from the two-stage regulator. The line is now purged, pressurized, and ready for use. Increase the pressure in the line to the desired operating pressure (e.g., 40–60 psig or 3–4 bar).

Purging Carrier and Makeup Gases Carrier-gas and makeup-gas systems require a static purge, followed by a dynamic purge, to ensure the desired purity levels.

STATIC PURGE Open all valves in the main and branch gas lines, but close the shutoff valves at the vent ends of the lines. Slowly open the valve on the cylinder, pressurizing the two-stage regulator. Slowly open the downstream pressure control knob on the regulator, allowing gas to flow through the lines. Raise the mainline pressure to the pressure you intend to maintain (typically 60–100 psig or 4–7 bar). Close the downstream pressure control valve on the two-stage regulator. Hold the system under pressure for 15 min, and then allow a very small flow to escape from the shutoff valves at the vent ends of the lines. The pressure will drop quickly. Close the shutoff valves just before the pressure reaches zero. It is important that these steps not take too long, or air could leak back into the system. Repeat this step 10 times. This allows impurities trapped in sections of the line to diffuse or desorb into the static purge gas.

DYNAMIC PURGE *After* the last static purge, close the shutoff valves at the vent ends of the lines and bring the mainline pressure to 20 psig (1.4 bar). Choose a shutoff valve as far downstream from the cylinder as possible. Open this valve and adjust the valve to allow a 60 mL/min flow of gas. Purge for 24 hs. For this step an extra flow controller, installed after the opened shutoff valve, will make it easy to regulate the flow and will help minimize backdiffusion into the line.

10.4.1.3 Purifier Connections

After all gas lines are purged, it is time to install and purge the purifiers you will be using. Most purifiers are factory-sealed with nitrogen or helium and will not contain air. Thus, these devices require only a short purge time before you can operate your system. Other purifiers may require many hours to purge. Read the instructions that accompany the purifiers you intend to use, to be sure that you purge them properly.

At this point all system components should be in place. The only part of the system left to purge is the short length of tubing connecting to the chromatograph.

Hereafter, whether the chromatograph is in use or idle, all lines should remain pressurized at all times.

10.4.2 Installation

Gas chromatograph installations range from simple single-chromatograph systems to very complex multibench systems. Concerns for a simple installation also are important for the complex multiple-instrument system. If you plan to design a complex system, you first should read and understand the information presented for the simpler systems, as well as the basic information in the first sections of this chapter. In designing any system, take time to consider your future needs.

Most plumbing problems develop when a change is made to an existing system. We recommend installing a gas chromatograph with valving and bypass fittings that will quickly allow you to add one or more gas chromatographs. Regardless of how many gas chromatographs are involved the operation, the addition or removal of any gas chromatograph from the system should not affect the operation of the other gas chromatographs in the system.

10.4.2.1 Single-Gas Chromatograph

Location One of the first steps should be to select a location for the chromatograph. Site selection is important for many reasons, including efficient functioning of both the chromatograph and the operator. Consider temperature and humidity. Generally, instrument manufacturers ask that the room air temperature is 20–27°C (68–80°F) and the relative humidity is 50–60% (with no condensation). Air exchange for the oven is very important to the operation of a gas chromatograph. The back of the instrument must be clear for at least 1 ft (30.5 cm). The gas chromatograph will be venting hot air from the oven to this area. This cannot be accomplished if the vent from one gas chromatograph is too close to a wall or the back of another instrument. Do not back instruments against one another, or against other heat-sensitive equipment, so that they vent toward each other. Special vent-directing devices can be installed to avoid these problems (consult your chromatograph manufacturer). Similarly, do not place the gas chromatograph by a window, or directly under air conditioning or other types of vents. Do not allow the paper from computers, integrators, or recorders to be exposed to the vents from ovens or other heated devices. This can cause a fire or, at the

least, discolor heat-sensitive paper. Ignoring these precautions can cause erratic temperature control, electrical problems, and shorter equipment life.

The operator will need room to store samples, along with syringes and other tools, before and after injection. It is best to leave at least a 2×2-ft working surface for this purpose. The instrument model will define the space requirement of the gas chromatograph, but in most cases, a 3-ft-wide space is adequate. Add two additional feet for computer controls and other ancillary devices (autosampler controls, purge-and-trap devices, sample concentrators, etc.). Thus, most gas chromatographs and associated devices will require about 6–8 linear feet of counter space. For most labs, this means no more than three to four gas chromatographs on a 20–24-ft (6–7-m) bench.

Gas Cylinders and Gas Lines Your next decision is to determine where to locate the one to six gas cylinders or the generators you will need to operate your chromatograph. Some facilities prohibit the storage of high-pressure cylinders in labs or hallways. Consult your safety department to determine a suitable location for your cylinders.

Ideally, you want the cylinders as close to the gas chromatograph as possible. The shortest length of tubing with the fewest connections is best. Never make a connection in a location that will be hard to access for leak testing (e.g., overhead, in a ceiling, or behind a bench that is against a wall).

The diameter of the gas line between the cylinder and the gas chromatograph depends on the distance. For a single-gas chromatograph with cylinders within a few feet of the instrument, $\frac{1}{16}$-in. (1.59-mm) stainless steel or $\frac{1}{8}$-in. (3.18-mm) copper tubing normally is used. However, these small-diameter lines can be only a few feet long, or backpressure will be high (see Table 10.9). If the cylinders are further away, $\frac{1}{4}$-in. (6.35-mm) tubing typically is used. Use $\frac{1}{2}$-in. (12.7-mm) tubing when the distance is extreme [$\geq$20 ft ($\geq$6 m)]. Reduce $\frac{1}{4}$-in. (6.35-mm) or $\frac{1}{2}$-in. (12.7-mm) mainlines to $\frac{1}{4}$-in. (6.35-mm) or $\frac{1}{16}$-in. (1.59-mm) tubing immediately before the connection to the chromatograph(s).

As a rule, we suggest using wider-bore tubing than a first evaluation would indicate. With larger lines, you have adequate pressure and flow for additional units, and will not have to redo the lines. Allow for expansion and you will save yourself much trouble in the future.

Simple Basic Plumbing for One-Gas Chromatograph The diagrams in Figures 10.23–10.28 show the various alternative recommendations for installing a single-gas chromatograph/FID, using gas cylinders, cylinders and generators, or generators, and using hydrogen as fuel or as fuel and carrier gas. TCDs, ECDs, and other detectors do not require a fuel gas line. Although we recommend gas purifiers as safeguards, the purifiers shown in Figures 10.23–10.28 can be removed if you do not think you need this additional protection. Similarly, intermediate shutoff valves in the line are useful but not vital. Be aware that simplifying your system by eliminating purifiers, shutoff valves, and other

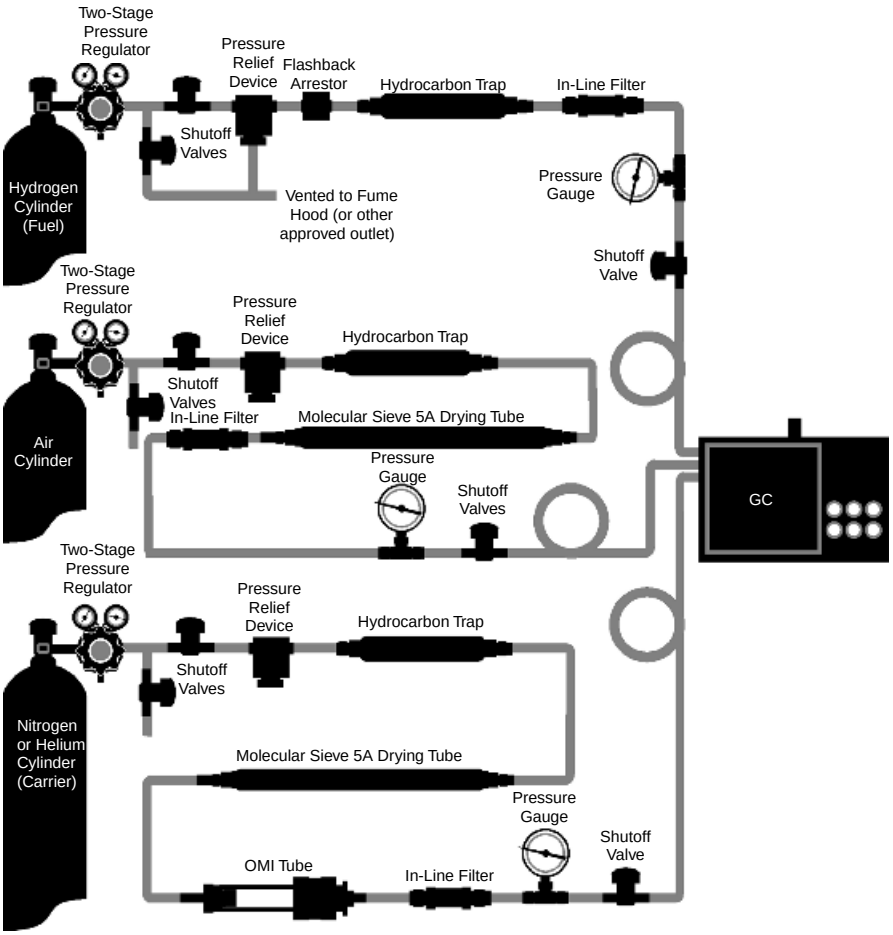
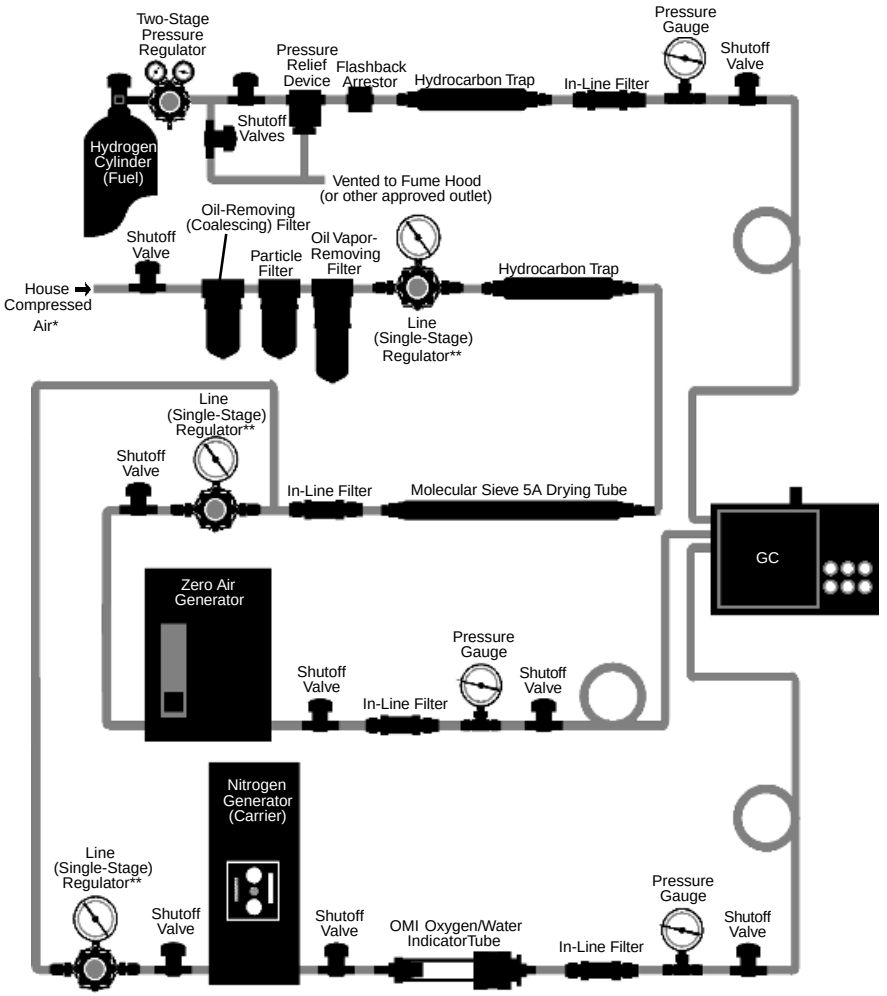


FIGURE 10.23 Ideal configurations for a single-gas chromatographic system: gas delivered from cylinders [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

components reduces initial costs, but you pay a higher price in terms of inconvenience (longer downtimes) and loss of column/detector protection.

The single-cylinder installations in Figures 10.23–10.25 leave you with the problem of having to cool down the gas chromatograph and slowly depressurize the entire system to change cylinders. Figure 10.28 shows a two-cylinder approach that can be used for continuous delivery of any gas. When the pressure in one cylinder indicates that the cylinder must be changed, the empty cylinder can be closed and the reserve cylinder opened. We recommend this approach. However, you should immediately take the time to change the empty cylinder or the extra plumbing will be for naught. If you do not change cylinders immediately, chances are good that you will forget to do so, both cylinders will be empty, and you will still have to shut down your gas chromatograph.



*Replace an oil-sealed compressor with an oilless unit to eliminate the need for the particle filter, oil-removing/coalescing filter, and oil vapor-removing filter.
**Consult generator manual for correct inlet pressure.

FIGURE 10.24 Ideal configurations for a single-gas chromatographic system: mixed-gas generator/gas cylinder system [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

A second approach to cylinder changeover also is viable. An automatic changeover regulator system (Figure 10.29) connects two gas cylinders: the active cylinder, and a reserve cylinder. When the pressure in the active cylinder falls below a preset level, gas automatically begins to flow from the reserve cylinder. You can change cylinders at your convenience without interrupting the analysis.

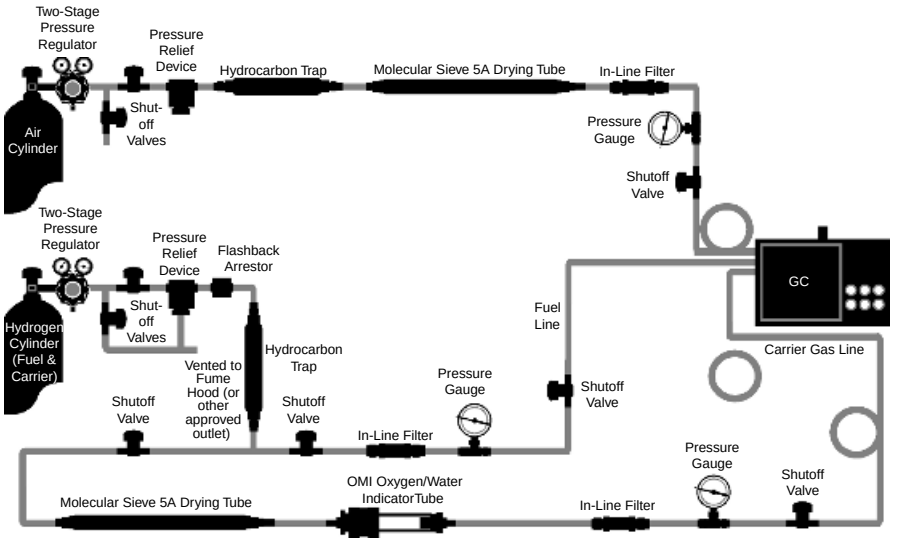
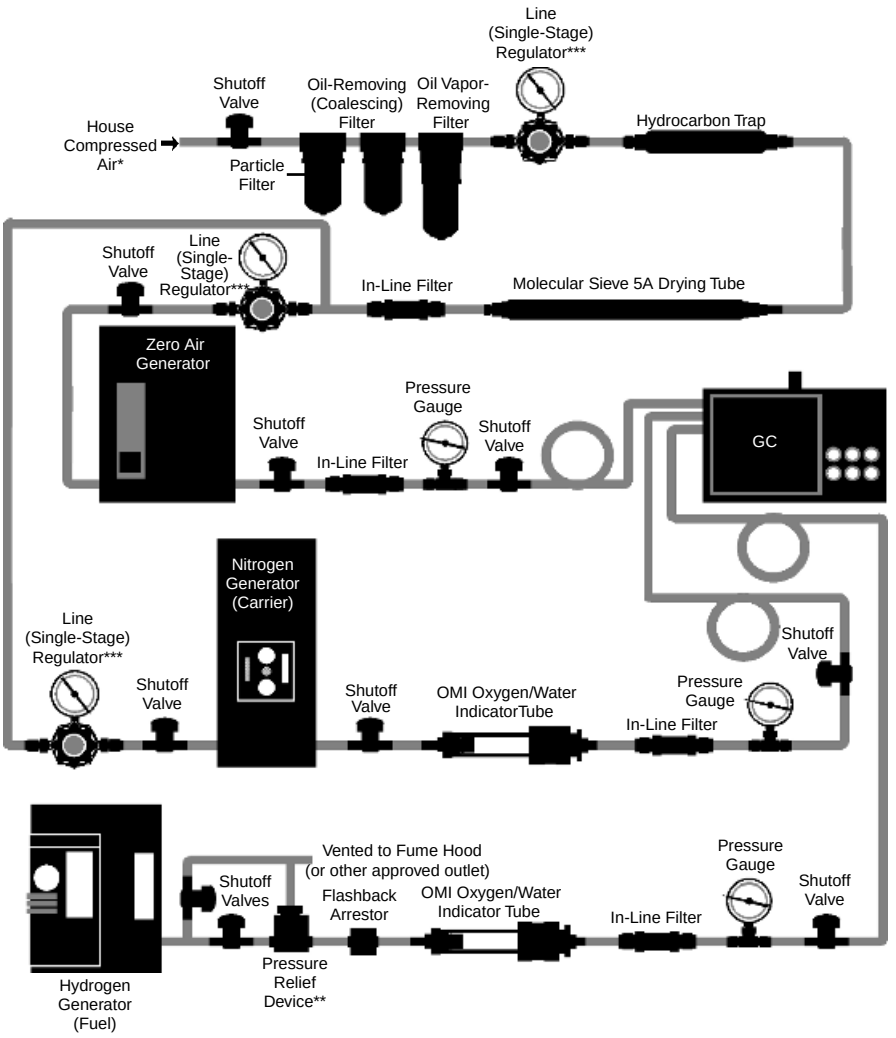


FIGURE 10.25 Ideal configurations for a single-gas chromatographic system: hydrogen used as carrier and fuel gas [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

The changeover regulator system works on a pressure differential. The line pressure from the active cylinder is set about 5 psig (0.4 bar) higher than the pressure from the reserve cylinder. Both cylinders are open, but the reserve cylinder will not deliver gas as long as the active cylinder can deliver gas at a pressure 5 psig (0.4 bar) higher than the pressure from the reserve cylinder. This approach requires two pressure regulators and a downstream inline regulator, or the gas chromatographs will register the change in pressure when the cylinders switch operation.

10.4.2.2 Two- to Four-Gas Chromatographs

When you plan to install a two to four gas chromatographic system, you must concern yourself with issues that did not arise with a single-gas chromatograph. Line diameters and connections, types of purification, valving, locations, and electrical needs all become more complicated. The plumbing changes from the relatively simple straight lines of tubing shown in Figures 10.23–10.28, to a complicated assortment of valves, fittings, and other components. Figure 10.30 shows a manifold system of three mainlines, adequate for two- to four-gas chromatographs. For each gas, a two-stage regulator controls gas pressures in the mainline and single-stage regulators are used in each branchline. We recommend that the mainline pressure be 90–100 psig (6–7 bar) and the individual line regulators be capable of providing up to 75 psig or 5 bar (see Figure 10.13). If your

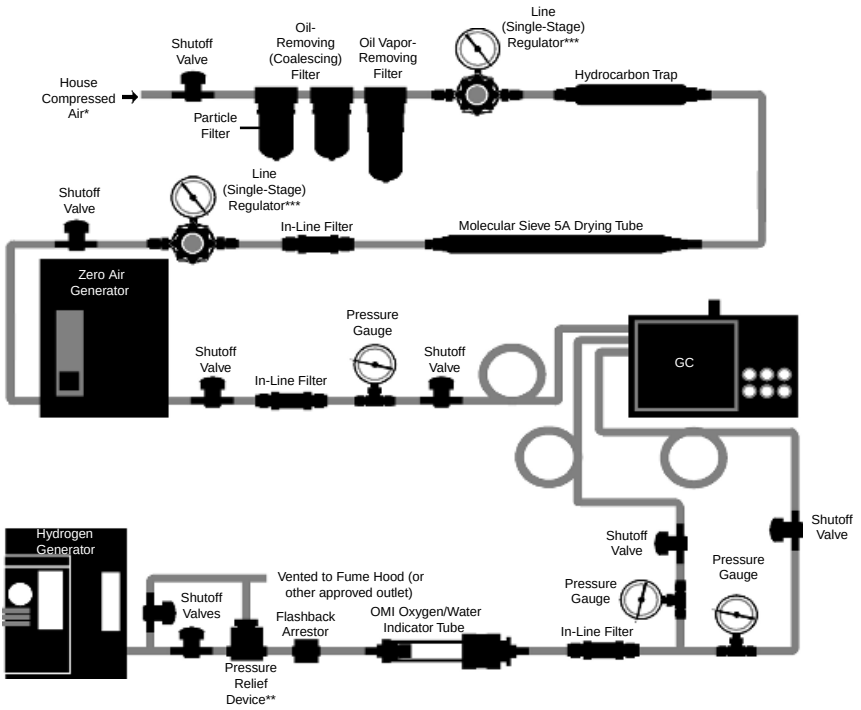


*Replace an oil-sealed compressor with an oilless unit to eliminate the need for the particle filter, oil-removing/coalescing filter, and oil vapor-removing filter.
**Not needed if the hydrogen generator has a built-in relief device.
***Consult generator manual for correct inlet pressure.

FIGURE 10.26 Ideal configurations for a single-gas chromatographic system: all-generator system [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

cylinders are located more than 20 ft (6.1 m) from the bench, you should use $\frac{1}{2}$-in. (12.7-mm) mainlines.

Note that there is a shutoff valve after each branchoff from the mainline. If your budget allows, we recommend installing these valves because they enable you to pressure-test individual sections of the system, or isolate each gas chromatograph



*Replace an oil-sealed compressor with an oilless unit to eliminate the need for the particle filter, oil-removing/coalescing filter, and oil vapour-removing filter.
**Not needed if the hydrogen generator has a built-in relief device.
***Consult generator manual for correct inlet pressure.

FIGURE 10.27 Ideal configurations for a single-gas chromatographic system: all-generator system with hydrogen as carrier and fuel gas [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

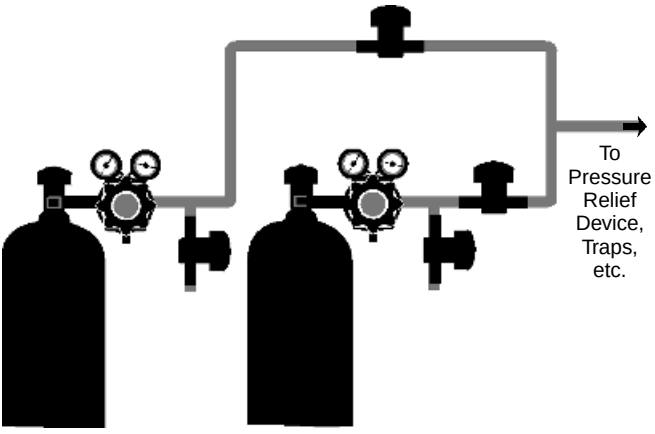


FIGURE 10.28 System using two cylinders for each gas [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

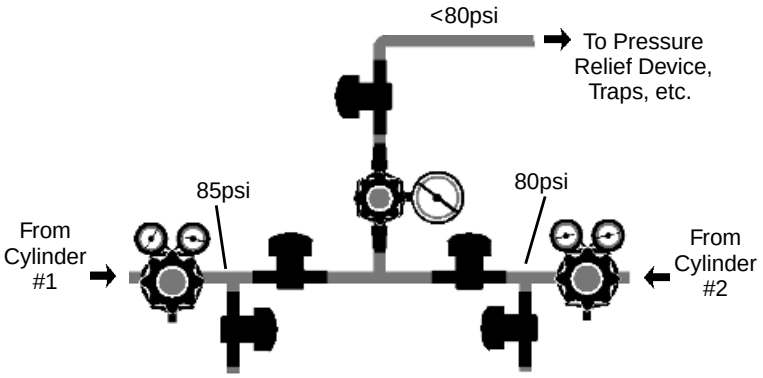


FIGURE 10.29 Automatic switchover manifold system [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

and take it offline without affecting the operation of the other gas chromatographs. The system also has inline pressure gauges after the two-stage regulators, to indicate the pressure in the mainlines.

In a multiple-instrument system, carrier-gas and makeup-gas purification also becomes more important. If you have only one gas chromatograph, you might not need gas purification, but with all the additional connections, regulators, and other devices in a system with four-gas chromatographs or less you almost certainly will need several types of purifiers.

Consider the total environment of your multigas chromatograph installation. All the integrator and detector cables, gas lines, and electrical power lines that you will need should be clearly labeled to allow easy access and identification; you should also consider interference with electrical signals. Allow access room at the front and back of each instrument.

Gas and electric lines should not be left to dangle; this can cause safety problems and confusion over what gas a line contains. Various mounting devices are available for gas and power lines, and these devices should be used. We recommend labels and color coding for the gas lines. Neatness does count.

Gas Cylinders or Gas Generators? With four-gas chromatographs or less you will need many gas cylinders. Carefully select the site for the cylinders. To keep the instruments running without interruption, you will not want to shut down instruments to change cylinders. Thus, you will need additional plumbing (i.e., a two-cylinder system, or an automatic changeover system, as shown in Figures 10.28 and 10.29). Furthermore, cylinder changes more frequent than once per week are an inefficient use of manpower. Calculate your gas consumption from the equation and example given in the installation information for 5–20 gas chromatograph systems. If you change cylinders frequently, more than once per week, consider using larger cylinders, cradles of cylinders, or gas generators. Cylinder cradles can be used allowing for a single connection to your system. The

location of labs in the centers of buildings often forces the cylinders-or-generators issue. Generators eliminate the need for very long gas lines or cylinders mounted in hallways. If you decide to use generators, allow bench or wall space for them, as near the gas chromatographs as possible.

Electrical Concerns Electrical requirements for installing two- to four-gas chromatographs are similar to those for a single-gas chromatograph. Each instrument should be on its own 15–20-A circuit. Try to keep related electrical devices (integrators, computers, etc.), except electrically actuated devices, on the same circuit. Detector and integrator cables need to be shielded and located 6 in. or more away from the electrical lines. The gas lines, particularly copper lines, should be 6–12 in. (15–30 cm) away from the power lines—they can pick up electrical current if they are too close to the power lines.

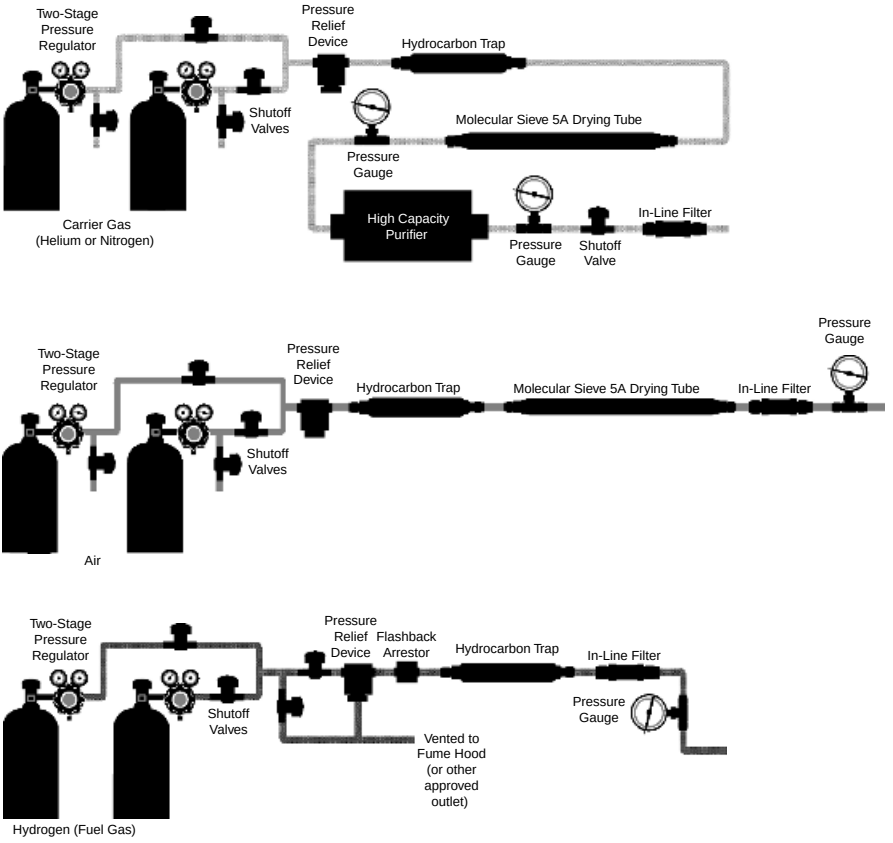


FIGURE 10.30 Ideal configurations for two- to four-gas chromatographic systems (plumb gas generators as shown in Figure 10.27) [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

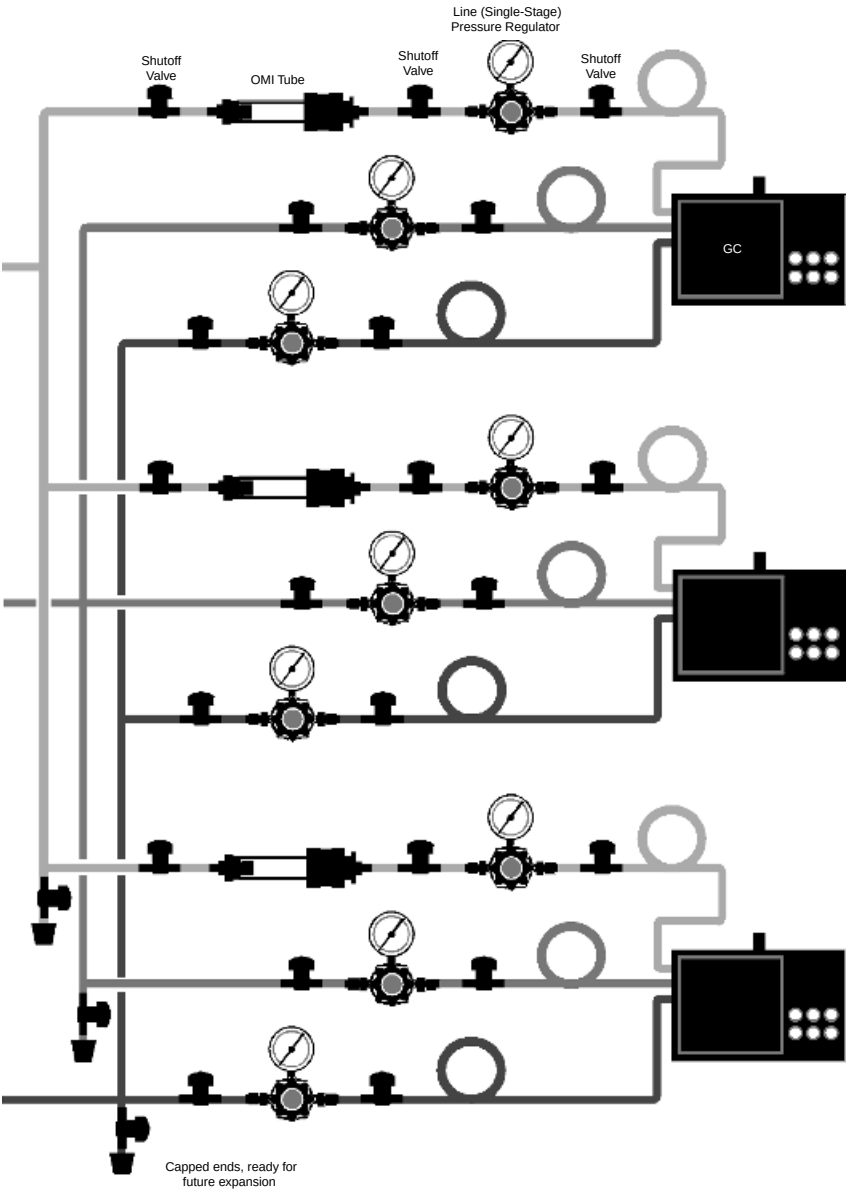


FIGURE 10.30 (*Continued*)

Of real concern is power interruptions that allow heated zones in the gas chromatographs to cool down. When the power is restored, these heated zones all come on together and have a tremendous power draw. When electricity goes off, it is best to turn off the main power switch for each gas chromatograph. When the electricity comes back on, turn each gas chromatograph back on by

zones: detector heaters, then the inlet, and then the oven. Consult an electrician about your power needs. Do not forget to establish separate earth grounding for your lines.

10.4.2.3 5–20-Gas Chromatographs

The additional major concerns for installing a laboratory of gas chromatographs deal with gas line diameters, gas flow measurement, and upsizing devices. First, you need to know how much gas the facility might use. Consider that each gas chromatograph with two flame-type detectors could use the amounts of gases listed in Table 10.2. Add the gas flows for all the gas chromatographs to obtain an estimate of the total gas use. After converting the volume from mL/min to standard cubic feet (SCF) per day, divide the volume of gas in one cylinder by the consumption per day. From this calculation, you can determine how long each cylinder should last. Determine this consumption for each of the gases you intend to use.

$$\frac{\text{Number of gas chromatographs} \times \text{flow} \times \text{min/day}}{\text{mL/SCF}} = \text{SCF/day} \qquad (10.3)$$

Days of usage per cylinder

$$\frac{\text{SCF/cylinder}^*}{\text{SCF/day}} = \text{days/cylinder} \qquad (10.4)$$

An example to determine the nitrogen consumption for five-gas chromatographs using 266 mL nitrogen/min per each gas chromatograph:

$$\frac{\text{Five-gas chromatographs} \times 266 \text{ mL/min} \times 1440 \text{ min}}{28{,}317 \text{ mL/SCF}} = 67.6 \text{ SCF/day}$$

$$\frac{28 \text{ SCF/cylinder}^*}{67.6 \text{ SCF/day}} = 3.2 \text{ days/cylinder}$$

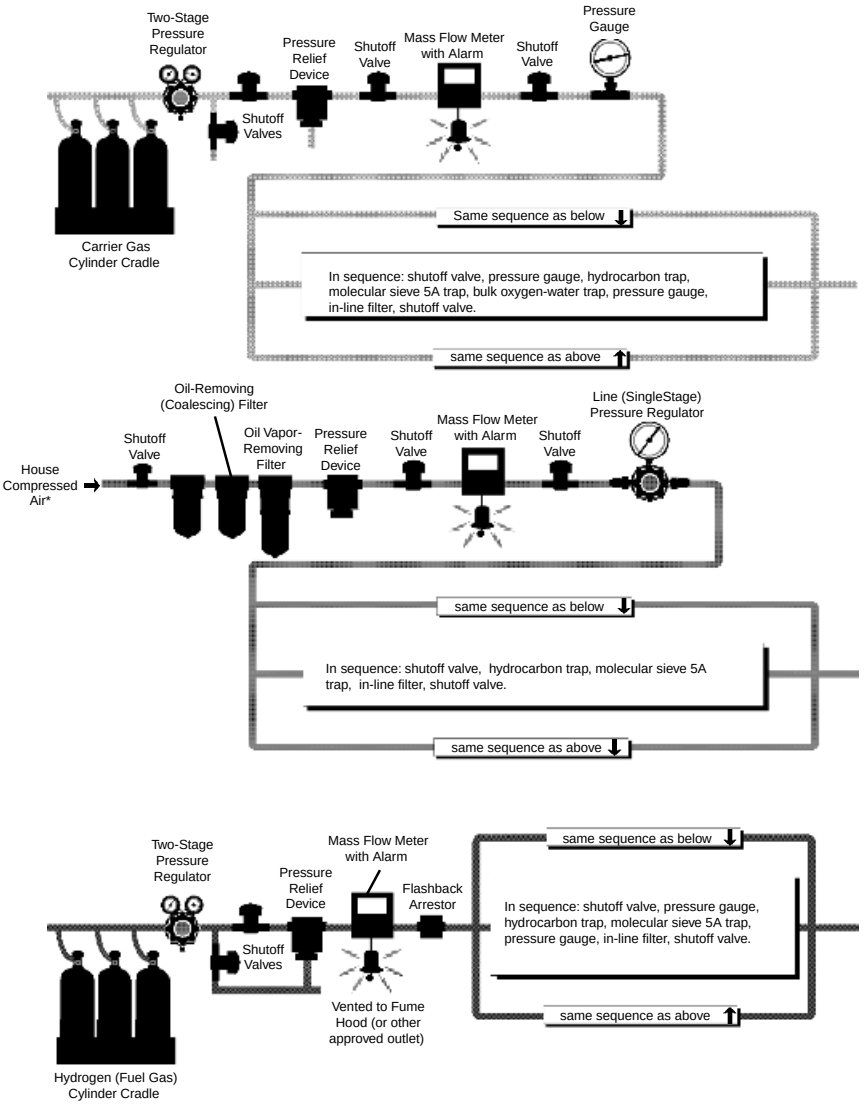
Rotameters In many large-scale gas chromatograph installations, rotameters are used as visual indicators of gas usage. If the rotameter is of the proper size, so that gas use per bench suspends the float or ball midway in the rotameter tube, a quick glance will tell you if you are using the correct amount of gas. Leaks tend to push the float off scale—leaks can easily more than double or more your gas consumption. We recommend one rotameter for the entire lab and one for each bench (Figures 10.31).

Mass flowmeters also are used in large facilities, to determine the total flow of gas into the facility. Often these devices provide an alarm if the flow is too high. A high flow might indicate a break somewhere in the lines. The use of

*Ask the cylinder supplier for specifications on your cylinder.

rotameters and mass flowmeters to monitor gas consumption can give very good information about the integrity of the gas systems, and can help you quickly find leaks.

Gas Purifiers With high gas usage, you can consume these devices rapidly. It might be necessary to mount several purifiers in parallel, to obtain reasonable life


Two-Stage Pressure Regulator
Pressure Relief Device
Shutoff Valve
Mass Flow Meter with Alarm
Shutoff Valve
Pressure Gauge
Shutoff Valves
Carrier Gas Cylinder Cradle
Same sequence as below
In sequence: shutoff valve, pressure gauge, hydrocarbon trap, molecular sieve 5A trap, bulk oxygen-water trap, pressure gauge, in-line filter, shutoff valve.
same sequence as above
Oil-Removing (Coalescing) Filter
Shutoff Valve
Oil Vapor-Removing Filter
Pressure Relief Device
Shutoff Valve
Mass Flow Meter with Alarm
Shutoff Valve
Line (SingleStage) Pressure Regulator
House Compressed Air*
same sequence as below
In sequence: shutoff valve, hydrocarbon trap, molecular sieve 5A trap, in-line filter, shutoff valve.
same sequence as above
Two-Stage Pressure Regulator
Mass Flow Meter with Alarm
Pressure Relief Device
Flashback Arrestor
same sequence as below
In sequence: shutoff valve, pressure gauge, hydrocarbon trap, molecular sieve 5A trap, pressure gauge, in-line filter, shutoff valve.
Shutoff Valves
Vented to Fume Hood (or other approved outlet)
same sequence as above
Hydrogen (Fuel Gas) Cylinder Cradle

*Replace an oil-sealed compressor with an oilless unit to eliminate the need for the particle filter, oil-removing/coalescing filter, and oil vapor-removing filter.

FIGURE 10.31 Complex systems in a large lab [reprinted with permission of Supelco, Bellefonte, PA 16823 (USA)].

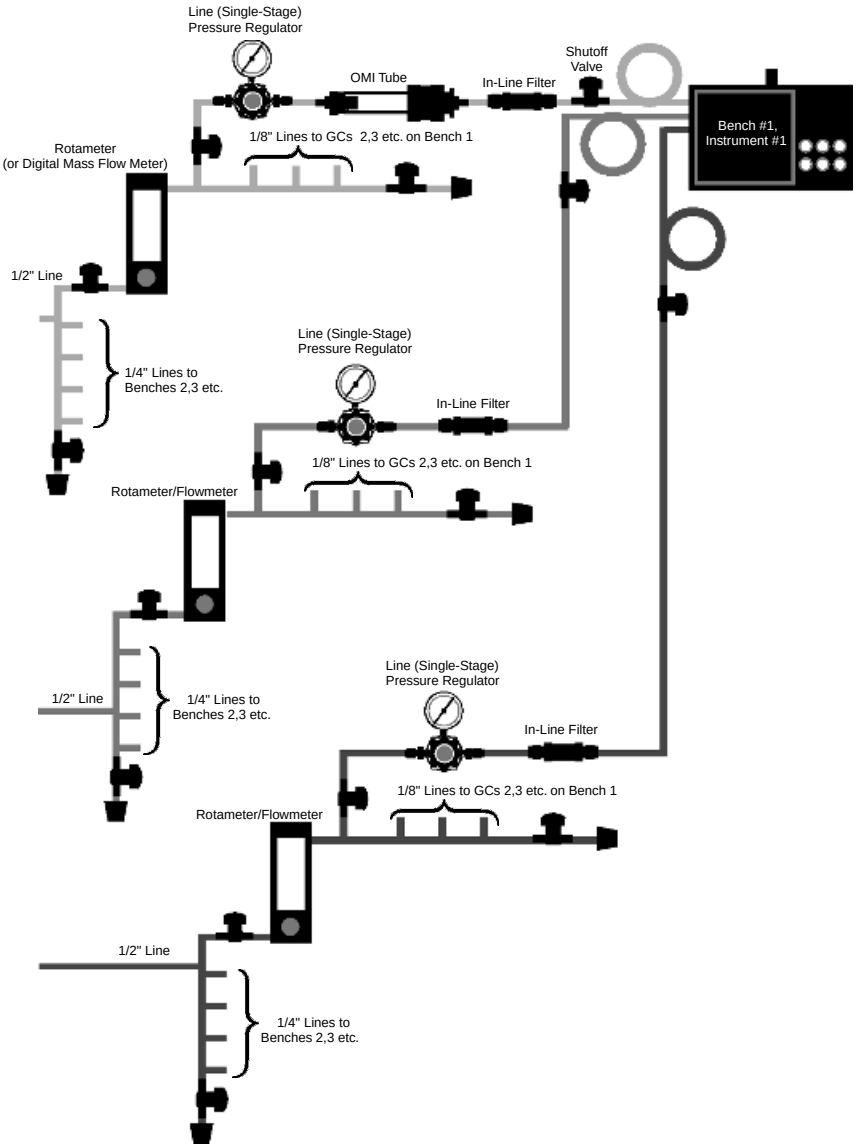


FIGURE 10.31 (*Continued*)

from the individual purifiers (Figures 10.30 and 10.31). Establish a maintenance program for regularly changing these purifiers. A more practical approach is to use larger purifiers. Hydrocarbon traps and molecular sieve–containing moisture traps with 750-μL adsorbent beds—3–5 or more times the capacity of conventional traps—are available. The $\frac{1}{4}$-inch (6.35-mm) or $\frac{1}{2}$-inch (12.7-mm) end fittings on these large traps are compatible with the larger-diameter gas lines

used in 5–20-gas chromatograph systems, minimizing the pressure drop across the traps. The large-capacity traps effectively remove contaminants at flowrates of up to 10 L/min.

Electrical Considerations Consult an electrical engineer about the special needs of a large gas chromatographic facility, explaining the need for separate, dedicated grounded lines for each gas chromatograph and associated equipment. As when designing simpler systems, estimate your total power needs by adding approximately 2100 W for each gas chromatograph, and the needs of the integrators and all peripheral equipment which you anticipate using.

TRADEMARKS

Crescent—Cooper Industries
Drierite—Hammond, W. A., Drierite Company
GateKeeper—Aeronex, Inc.
Glasrench, OMI, Supelcarb, Supelpure—Sigma-Aldrich Co.
Hall—Tracor Instruments, Austin, Inc.
Imp—Gould, Inc., Valve & Fittings Div.
Leak-Tec—American Gas & Chemical Co., Ltd.
MAPP—Dow Chemical Co.
Nanochem—Matheson Gas Products
Nupro, Snoop—Nupro Co.
Oxiclear, Oxisorb—MG Industries
Teflon—E. I. du Pont de Nemours & Co., Inc.
Viton—DuPont Dow Elastomers
Whitey—Whitey Co.

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PART III

Applications

Science is nothing but trained and organized common sense, differing from the latter only as a veteran may differ from a raw recruit. And its methods differ from those of common sense only as far as the guardsman's cut and thrust differ from the manner in which a savage wields his club.

—Thomas Henry Huxley (1825–1895)
Collected Essays, iv, The Method of Zadig

CHAPTER ELEVEN

Sample Preparation Techniques for Gas Chromatography

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11.1 INTRODUCTION

Modern gas chromatography cannot be fully treated without also discussing sample preparation. Unlike most other instrumental techniques, gas chromatography requires many specialized sample preparation techniques, due to the requirement that samples for GC be vaporized in the inlet. Further, in most cases, the analytes must be distributed in an organic liquid or a vapor phase prior to injection. The myriad sample matrices and interferences that may be present further complicate this. As a result, there are a tremendous variety of sample preparation techniques available for gas chromatographers. These range in complexity from simple dilutions and injection of "neat" samples, to sophisticated fully on-line instruments such as supercritical fluid extractors.

In this chapter, we will provide a basic overview of the principles, practice, and applications of the major sample preparation techniques for gas chromatography. Most are based on variants of extraction theory, so a theoretical overview of liquid and vapor phase extraction theory is also presented.

11.1.1 Types of Samples for Gas Chromatography

The sample that is injected into the gas chromatograph following sample preparation must be either a liquid or a gas, the analytes must be volatile enough under the conditions of the inlet and column to traverse the instrument, and, ideally, the matrix interferences must also be volatile, so as not to contaminate the instrument or column. In most cases, liquid samples must be dissolved in a volatile organic solvent. The basic goal of sample preparation is to ensure that these conditions are met, with additional goals that the preparation be reproducible to meet quantitative analysis requirements and straightforward to perform, if the analysis is to be performed routinely, as in quality assurance and in other routine testing laboratories.

Very few native analytical samples, which may be solids, liquids, gases, or simple or complex mixtures and contain volatile and nonvolatile contaminants, meet these requirements. Table 11.1 provides an overview of the sample preparation techniques described in this chapter, sorted by the phase of the bulk sample. It is readily seen that there are numerous possibilities possible for a given sample type. This presents the choice of sample preparation technique as one of the most difficult choices in developing an analysis. Most interesting among these, is the possibility of changing the phase of the sample (by dissolving in a solvent or trapping on a sorbent, for example) to make the sample more amenable to available, sensitive, or selective instrumentation.

11.1.2 Fundamentals of Extraction Theory

Most of the techniques listed in Table 11.1 and described in this chapter involve some form of extraction to remove the analytes of interest from the sample matrix

TABLE 11.1 Overview of Sample Preparation Techniques by Sample Type

Solid	Liquid	Gas
Dissolving followed by liquid technique	Direct “neat” injection	Direct “neat” injection (syringe or sample valve)
Supercritical-fluid extraction	Liquid–liquid extraction	Membrane extraction
Headspace extraction	Solid-phase extraction (includes SPME, sorbent-based extractions)	Trapping on a solid followed by solid technique
Accelerated solvent extraction	Headspace extraction (includes SPME, sorbent-based extractions)	Trapping in a liquid followed by liquid technique
Pyrolysis	Membrane extraction	—
Thermal desorption	Trapping on a solid followed by solid technique	—
Microwave-assisted extraction	—	—

and to place those analytes into a phase that is amenable to injection. While the specific details of each extraction technique are left to the individual discussions, they are all rooted in the fundamentals of extraction theory that was classically developed for liquid–liquid and liquid–vapor extraction equilibria. These theories also provide the basis for chromatographic retention theory, which is presented in Chapter 2.

11.1.2.1 Theory of Liquid–Liquid Extraction

Liquid–liquid extraction is perhaps the most classical of all sample preparation techniques, as it is taught as the early stages of most chemistry students' careers (1). The fundamentals of liquid–liquid extraction provide a background for all other extraction techniques described in the literature. In liquid–liquid extraction, dissolved components are transferred from one liquid phase to another. Most commonly in GC, this is performed to transfer analytes from an aqueous phase to an organic phase that is more amenable to gas chromatographic analysis. The main requirement is that the two liquid phases be completely immiscible.

In considering a system containing more than one phase, the phase rule, which derives from the second law of thermodynamics and stated below in its most recognizable form, must be applied

$$F = C - P + 2 \tag{11.1}$$

where F is the number of degrees of freedom (the number of variable factors that must be fixed to completely define a system at equilibrium) that must be considered, C is the number of components present, and P is the number of phases present. In the case of a liquid–liquid extraction, in which one analyte is to be transferred between two immiscible liquids, there are three components (the analyte plus the two liquids) and two phases, giving 3 degrees of freedom: temperature, pressure, and concentration.

The distribution of a solute between two immiscible liquids, which ultimately governs the ability of the system to extract the solute from one liquid to the other, is given by the distribution law, originally stated by Berthelot and later by Nernst

$$K = \frac{C_2}{C_1} \tag{11.2}$$

where K is the ratio of the concentration of the solute in phase 2 to the concentration of solute in phase 1. At a given temperature, K will be constant. If the system is then allowed to come to equilibrium, the equilibrium chemical equation may be described as

$$\text{A(phase 1)} \Leftrightarrow \text{A(phase 2)} \tag{11.3}$$

where A refers to the analyte. The equilibrium expression, defining the equilibrium distribution constant is given by

$$\tilde{K}_c = \frac{[A_2]}{[A_1]} \tag{11.4}$$

and the more rigorous thermodynamic equilibrium constant is defined by

$$K^0 = \frac{[A]_2\gamma_2}{[A]_1\gamma_1} \tag{11.5}$$

where γ is the activity coefficient for the solute in the given phase. Generally, in analytical chemistry, the less rigorous equilibrium distribution constant is used, as solutions are usually dilute enough to assume ideal behavior and activity coefficients of one.

In equilibrium constant expressions, concentrations are usually expressed as molar quantities. This is often impractical, so it is useful to express the distribution constant in terms of weights and volumes, which are often much simpler to measure.

$$K_c = \frac{(W_A)_2/MW_A}{V_2} \div \frac{(W_A)_1/MW_A}{V_1} = \frac{(W_A)_2}{(W_A)_1} \cdot \frac{V_1}{V_2} \tag{11.6}$$

where W_A is the weight of the analyte in grams, MW_A is the molar mass of the analyte in grams per mole, and V_1 and V_2 are the volumes of the two liquid phases. The capacity factor is defined as the weight ratio of the analyte in phase 2 to phase 1

$$k = \frac{(W_A)_2}{(W_A)_1} = \frac{[A]_2V_2}{[A]_1V_1} \tag{11.7}$$

where k is the capacity factor. This definition is identical to the physical definition of the chromatographic retention factor, presented in Chapters 1 and 2, and provides the connection between the theories of extraction and of retention in chromatography. Further, the definition of the phase ratio, a critically important quantity in gas chromatography, is seen as

$$\beta = \frac{V_1}{V_2} \tag{11.8}$$

If Equations 11.7 and 11.8 are combined, another expression, also shown in Chapter 2, is obtained

$$K_c = k\beta \tag{11.9}$$

which defines the distribution constant as the product of capacity factor and phase ratio for extraction and defines the partition coefficient as the product of retention factor and phase ratio in chromatography.

11.1.2.2 Extraction Efficiency

Commonly, the efficiency of an extraction process is defined based on the fractions of extracted and unextracted solute. For a given phase, the fraction of solute within the phase can be written as

$$\phi_2 = \frac{[A]_2V_2}{[A]_1V_1 + [A]_2V_2} \tag{11.10}$$

where ϕ_2 is the fraction of solute in phase 2. Combining with Equation 11.4 yields

$$\phi_2 = \frac{K_c V_2}{K_c V_2 + V_1} = \frac{K_c (V_2/V_1)}{K_c (V_2/V_1) + 1} \tag{11.11}$$

If Equation 11.11 is combined with Equation 11.7 and rearranged, then

$$\phi_2 = \frac{k}{k+1} \tag{11.12}$$

is obtained. By similar algebra the fraction remaining unextracted is obtained by

$$1 - \phi_2 = \frac{1}{1+k} \tag{11.13}$$

Since the capacity factor itself is a measured or derived quantity, it is important to further derive and obtain the fractions extracted and unextracted as functions of the distribution constant, which is more closely related to fundamental physical properties. It follows that the capacity factor is therefore equal to the ratio of extracted to unextracted analyte:

$$k = \frac{\phi_2}{1 - \phi_2} \tag{11.14}$$

Combining with Equation 11.9 and solving for ϕ_2 gives

$$\phi_2 = \frac{K_c V_1}{V_2 + K_c V_1} \tag{11.15}$$

which expresses the fraction of analyte extracted as a function of the distribution constant and the volumes of the two phases. By similar algebra, the fraction unextracted is given as

$$1 - \phi_2 = \frac{V_2}{V_2 + K_c V_1} \tag{11.16}$$

If the phase volumes are equal, Equations 11.15 and 11.16 reduce to

$$\phi_2 = \frac{K_c}{1 + K_c} \tag{11.17}$$

and

$$1 - \phi_2 = \frac{1}{1 + K_c} \tag{11.18}$$

It is seen that for liquid–liquid extraction and for any extraction technique based on equilibrium theory, that the values of the distribution constant and the volumes of the two phases are the critical factors in determining the fraction of analyte that is extracted. If multiple analytes are present, then the selectivity of the extraction derives from differences in the distribution constants.

11.1.2.3 Efficiency of Multiple Extractions

The derivations shown above may be applied to determining the maximum efficiency that may be obtained from an extraction. Efficiency may be described on the basis of the fraction of solute remaining in the original phase, termed the *raffinate* following successive equilibrations. An extraction is considered 100% efficient if all the analyte is transferred from the original phase (phase 1) to the extracting phase (phase 2). The fraction remaining in the original phase after a single extraction is given as

$$\phi_R = \frac{V_1}{K_c V_2 + V_1} \tag{11.19}$$

where V_1 is the volume of the original phase containing the solute and V_2 is the volume of the extracting phase. The fraction that was extracted into the extracting phase would then be given by

$$\phi_E = 1 - \phi_R \tag{11.20}$$

If n extractions are performed, with fresh extractant each time on the same original phase, then the fraction of analyte remaining in the original phase is given by

$$\phi_R^n = \left[\frac{V_1}{K_c(\sum V_2/n) + V_1}\right] \tag{11.21}$$

which shows that the fraction remaining in the original phase will always be less than 1, while the total fraction extracted will also always be less than one. In short, even with multiple extractions, extraction is never 100% efficient.

Figure 11.1 shows a plot of fraction extracted versus number of extractions for several values of K_c, assuming that the two phase volumes are identical. For low values of K_c, it is seen that exhaustive extraction is not practical; however, it is likely that enough analyte may be extracted to still be analytically useful. This

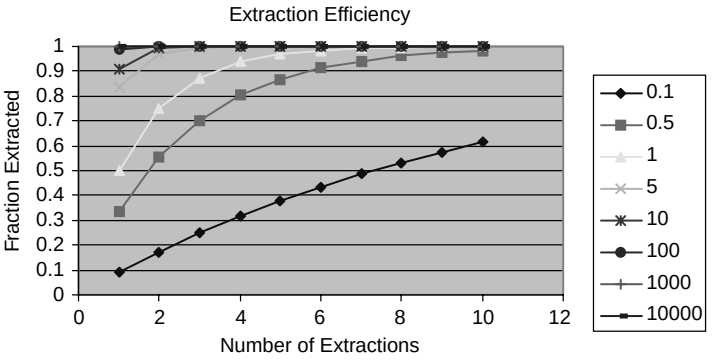

FIGURE 11.1 Plot of extraction efficiency versus number of extractions for values of K_c from 0.1 to 10000.

idea will be explored further in the discussion of several of the other extraction methods. It is especially interesting to note that continuous extractions techniques such as Soxhlet, dynamic headspace, and dynamic supercritical-fluid extraction (SFE) can be viewed as potentially unlimited multiple extractions, providing the possibility of quantitative extraction, even when K_c is low. At high values of K_c, (greater than $\sim$100), it is seen that nearly exhaustive extraction is achieved with one or two extraction steps. In most cases, not more then three to five extraction steps are generally needed to accomplish quantitative extraction.

11.2 PRACTICE OF LIQUID–LIQUID EXTRACTION

Despite the proliferation of other sample preparation techniques, liquid–liquid extraction remains the most commonly used technique in routine applications. The basic theory of liquid-liquid extraction has been discussed in Section 11.1.3 and can be extended to the analytical practice of preparing samples for gas chromatography. In most cases, this will involve the extraction of analytes from a dilute (concentration levels from part per trillion to part per thousand) aqueous phase into an organic phase. Often, for trace (100 ppm and below) analyte concentrations, the procedure also will include a concentration step, to improve sensitivity. Liquid–liquid extractions may be classified as classical or macroextractions, or microextractions, depending on the volume of extraction solvent used, with the dividing line at about 1 mL of extraction solvent. Classically, macroliquid–liquid extraction is performed using a separatory funnel, or a continuous extraction device. The main advantages of macroscale liquid–liquid extraction are simplicity and potential for concentration of trace samples. The main disadvantages are difficult automation and large amounts of high-purity solvents that must be consumed and disposed. Microscale liquid–liquid extraction is often performed using a volumetric flask, conical test tube, or directly in a sample vial, and often trades some of the potential concentration benefit for simpler automation and less solvent consumption. The choice of glassware most often depends on the convenient containment, separation and removal of the two phases.

11.2.1 Macroscale Liquid–Liquid Extraction

Macroscale liquid–liquid extraction usually refers to extractions in which more than a few milliliters of organic solvent are used. Commonly, large organic volumes are needed when the samples are very dilute (ppb levels and lower), therefore requiring concentration prior to gas chromatographic analysis. Liquid–liquid extraction may be carried out statically, as in the classical separatory funnel techniques, or continuously, in which organic solvent droplets are continuously passed through the aqueous phase and recycled by distillation. Continuous extraction suffers from the shortcoming that volatile analytes may be recycled back into the aqueous phase, limiting efficiency.

11.2.1.1 Soxhlet Extraction

Extractions involving transfer of analytes into an organic solvent are not limited to liquid samples or solutions. If a solid sample is to be analyzed, then Soxhlet extraction is the classical technique. In Soxhlet extraction, the solid sample is placed in a porous thimble above a solvent reservoir. As the solvent is heated, distilled solvent drips into the porous thimble, immersing the solid sample. When the thimble is full, solvent is siphoned back into the solvent reservoir and redistilled. If the sample particles are wet or hydrophilic, they may repel the organic solvent, reducing efficiency. Drying agents are often used to reduce this problem. Soxhlet extraction is generally used for semi- or nonvolatile analytes as volatiles may be lost through the condenser. A further disadvantage is that Soxhlet extraction is usually slow, often requiring several hours. This is often pointed out when Soxhlet extraction is compared to instrumental techniques such as supercritical fluid extraction or accelerated solvent extraction.

11.2.2 Microscale Liquid–Liquid Extraction

Liquid–liquid extractions involving a few milliliters or less of organic solvent are termed microscale liquid–liquid extractions. Very often, these can be carried out directly in autoinjector vials, thereby saving time-consuming and error-producing concentration and transfer steps. As an example of the flexibility of the various extraction techniques, Figure 11.2 compares extraction efficiencies for several possibilities. Extraction efficiency, with a value of 1 indicating exhaustive extraction, is plotted against the distribution constant. *MLLE* refers to a microscale liquid extraction carried out with equal volumes of solvent and sample in an autoinjector vial. *SPME* refers to solid-phase microextraction, which is described in Section 11.5.1 of this chapter. *High* refers to a macroscale liquid–liquid extraction with a high degree of concentration, and *low* refers to a macroextraction with

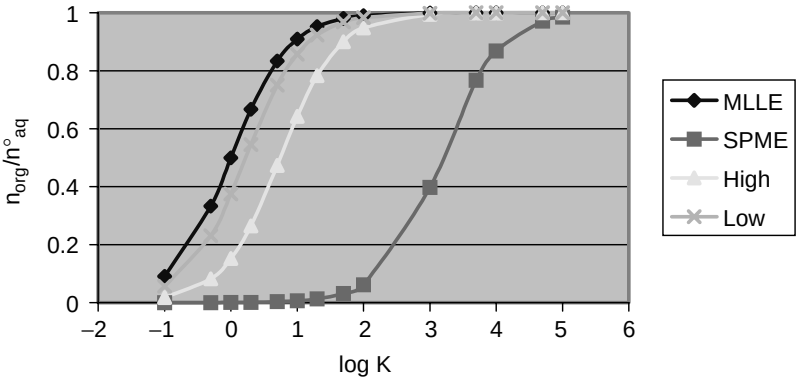

FIGURE 11.2 Comparison of extraction techniques. MLLE: 1-mL sample, 1 mL solvent; SPME: solid-phase microextraction; high—1-L sample, 3 × 60-mL solvent; low—5-mL sample, 3 × 1-mL solvent.

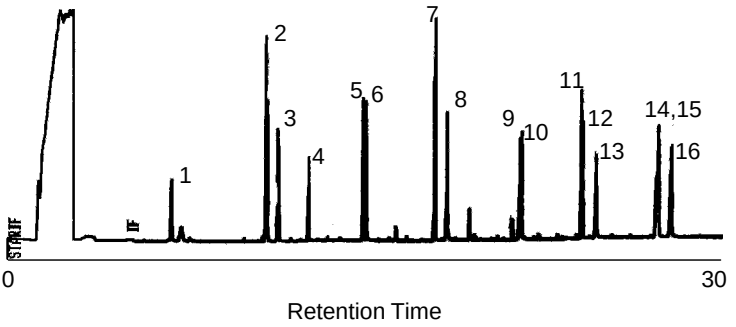

FIGURE 11.3 Separation of polycyclic aromatic hydrocarbons extracted using MLLE. GC: 5890 (Agilent), FID, injection—large-volume injection optic 2 (ATAS USA), Supelcoport packed liner, 50 μL injection, vent 1 min at 50°C, inlet TP: 8°C/s to 280°C and hold; column 30 m × 0.25 mm × 0.25 μm DB-5MS. Column TP: 50°C/1 min, 10°–300°C and hold [1 = naphthalene, 2 = acenaphthylene, 3 = acenaphthene, 4 = fluorine, 5 = anthracene, 6 = phenanthrene, 7 = fluranthene, 8 = pyrene, 9 = benzo(*a*)anthracene, 10 = chrysene, 11 = benzo(*b*)fluoranthene, 12 = benzo(*k*)fluoranthene, 13 = benzo(*a*)pyrene, 14 = indeno(1,2,3-*cd*)pyrene, 15 = dibenzo(*a*,*h*)anthracene, 16 = benzo(*g*,*h*,*i*)perylene].

a low degree of concentration. It is seen that it is readily possible for MLLE to be competitive with larger volume extractions, especially if employed in combination with large-volume gas chromatographic injection, as shown in Figure 11.3. This figure shows the separation of polycyclic aromatic hydrocarbons, extracted from aqueous solution and injected using programmed temperature vaporization large volume injection.

11.2.3 Single-Drop Microextraction

Carrying the idea of minimizing the volume of organic solvent in a liquid–liquid extraction to the extreme is a new family of techniques: single drop micro–extraction. The concept is simple: a single drop of organic solvent is suspended into the aqueous phase and the system is agitated to drive organic analytes into the drop. The organic drop can then be transferred to the gas chromatograph. Instrumentation for single-drop micro-extraction was introduced nearly simultaneously by Liu and Dasgupta (2) and Jeannot and Cantwell (3) in 1996. In the Jeannot–Cantwell system, a small drop (about 8 μL) of organic solvent containing an internal standard was suspended from the end of a Teflon rod into a stirred aqueous solution. Using a microsyringe, the organic drop was then transferred to a gas chromatograph for analysis. Figure 11.4 shows an improved design, first described in 1997 (4) in which the organic drop is suspended directly from a common gas chromatographic microsyringe.

The equilibrium theory of single-drop microextraction is similar to that seen in classical liquid–liquid extraction; the equilibrium concentration of analyte in

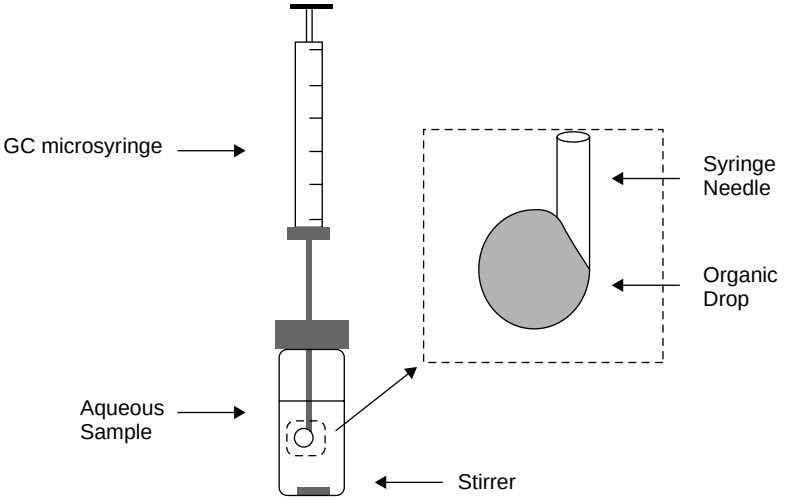

FIGURE 11.4 Schematic of a single-drop microextraction system using a GC syringe [reprinted with permission from E. Psillakis, and N. Kalogerakis, *TrAC Trends Anal. Chem.* **21**, 53 (2002), Figure 3].

the organic phase at equilibrium is given by

$$[\mathrm{A}]_2 = \frac{K_\mathrm{c}[\mathrm{A}]_1 V_1}{V_1 + K_\mathrm{c} V_2} \tag{11.22}$$

where the subscripts 1 and 2 refer to the aqueous and organic phases, respectively. As with other liquid–liquid extraction techniques, the addition of salt to the aqueous phase may affect the equilibrium position. Although salt addition commonly increases the amount extracted, the opposite has been observed with single-drop microextraction (5–7). This has been attributed to the higher ionic strength of the aqueous phase decreasing the analyte diffusion rate enough to offset the classical "salting out" effect.

The time required for the system to come to equilibrium is a critical factor in single-drop microextraction. Jeannot and Cantwell have studied the kinetics of single-drop microextraction and have found the strongest effect to be stirring rate of the aqueous phase, with more rapid stirring generating faster kinetics. Typical equilibration times range from 5 to 10 min. As a method development example, Figure 11.5 shows extraction recovery for 4-methylacetophenone from water into octane using single-drop microextraction at several different stirring rates, versus stirring time (4). These curves show classical extraction behavior, with equilibrium essentially reached in about 5 min. Further faster stirring promotes more rapid equilibration; however, it is more likely that the drop will fall off the syringe needle tip. Psillakis and Kalogerakis reviewed recent developments in single-drop microextraction (8).

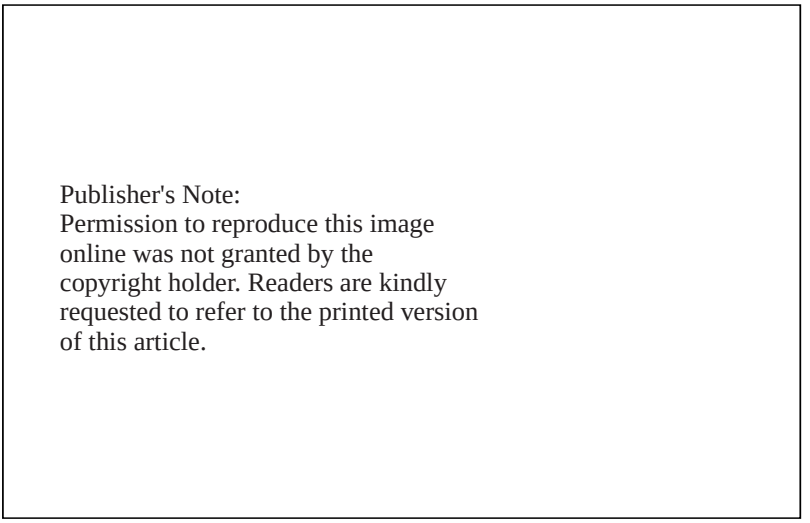
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FIGURE 11.5 Single-drop microextraction recovery versus stirring time (squares = 900 rpm, diamonds = 1200 rpm, triangles = 1500 rpm, circles = 1800 rpm) [reprinted with permission from M. A. Jeannot and F. F. Cantwell, *Anal.Chem.* **69** (1997), p. 235, Figure 2, copyright 1997, American Chemical Society].

11.3 SOLID-PHASE EXTRACTION

The first successful attempts to characterize organic analytes present in water, by trapping them on a carbon-based sorbent and eluting them with an organic solvent, were reported in the 1950s (9). The use of commercial solid phase extraction columns (SPE) to trap analytes was introduced in the late 1970s. Since that time, their use has grown rapidly, specifically the use of silicagel-bonded phases (10). Solid-phase extraction continues to be one of the most popular sample preparation techniques, owing to its low cost, ease of use, and excellent quantitation.

11.3.1 Basic Principles of Solid-Phase Extraction

Solid-phase extraction applies the principles of liquid chromatography to trap an analyte on a solid sorbent from a liquid matrix for concentration, cleanup, or phase exchange prior to analysis (11). The analytes, solvated in a weak solvent such as water, are trapped on a solid sorbent under conditions of high capacity factor and then eluted with a small volume of strong solvent with a high capacity factor (12). Figure 11.6 illustrates this principle. Whatever retention mechanism is chosen (normal phase, reversed phase, and ion exchange are most common), analytes are adsorbed under conditions of low solvent strength and high retention and then eluted under conditions of high solvent strength and low retention. The mechanism for SPE is similar to that of liquid–liquid extraction (LLE). For

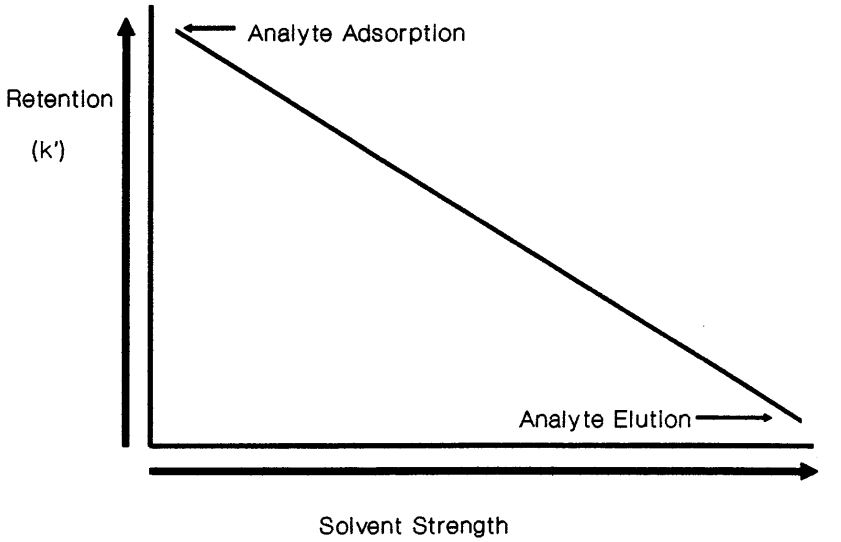

FIGURE 11.6 Graph of retention versus elution strength for a solid-phase extraction sorbent.

both SPE and LLE the distribution constant (K) of the analyte between solid sorbent (organic phase) and the aqueous matrix determines the amount of analyte extracted (13). For strongly hydrophobic compounds where the partition ratio is $>10^3$, nearly 100% of the analyte will be adsorbed onto the sorbent. For semipolar compounds, the analytes will have a greater affinity for the aqueous matrix, which results in a less favorable partition ratio and lower recoveries. However, these low recoveries can be overcome by taking the precautions described in the following sections.

11.3.2 Comparison between Solid-Phase Extraction (SPE) and Liquid–Liquid Extraction (LLE)

A typical SPE cartridge is depicted schematically in Figure 11.7. Most of these look very similar to filter cartridges, except that filter material is replaced by the sorbent. Solid phase extraction may also be performed using impregnated filter disks or syringe cartridges. These cartridges are designed to work with common vacuum manifolds, with manifolds capable of holding 10–50 cartridges readily available. Solid-phase extraction has replaced LLE in many laboratories for the following reasons:

1. SPE has eliminated the need to deal with the large volumes of organic solvent (14).
2. SPE gives the analyst the ability to sample in the field (15). Since large volumes of the aqueous sample can be passed through the SPE cartridge,

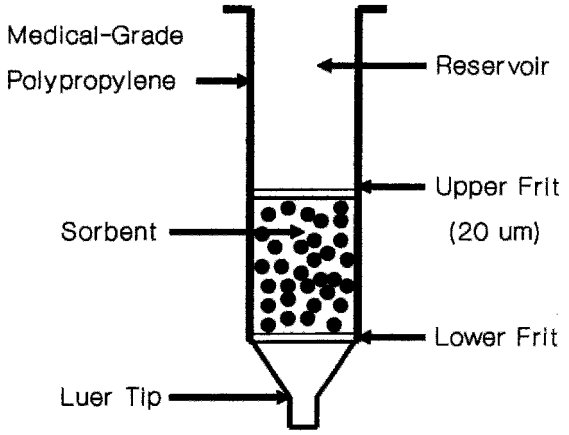

FIGURE 11.7 Schematic of a solid-phase extraction cartridge. Typical dimensions for a 3-mL cartridge are 5.5 × 1 cm. Samples are poured into the top of the cartridge and are drawn through the sorbent by vacuum or pressure.

preconcentration of analyte can be performed in the field, eliminating the need to transport liters of liquid in glass bottles back to the testing lab. The analyst only needs to transport the small SPE cartridges.

3. Using SPE is simple and can be preformed using a single SPE cartridge. If the initial SPE attempts are successful, then more sophisticated multiple cartridge systems can be employed.
4. Emulsion formation, which is one of the greatest drawbacks of LLE for wastewater and biological samples, is rarely a problem.
5. By reducing the amount of organic solvent necessary to complete the extraction, the risk of exposure to hazardous solvents is minimized.
6. SPE cartridges are relatively inexpensive, around a few dollars per cartridge, and this combined with the reduced solvent cost makes SPE 5–10 times less expensive than LLE (16). Although SPE cartridges are considered disposable, they can be regenerated and reused if the samples are not overly contaminated.
7. SPE cartridges are made from medical-grade polypropylene; therefore the contamination that occurs from poorly cleaned glassware, is unlikely.
8. The flexibility of SPE exceeds that of LLE. The solvents available for use in SPE are almost limitless, while LLE is limited to extremely hydrophobic solvents only. In addition, the wide selection of SPE sorbents provides the ability to maximize selectivity (α) (17).
9. SPE method development is straightforward. Often the analyst can find a method of extraction in the application notes provided by SPE vendors (18,19) or by having knowledge of previously developed HPLC methods for analysis of the compounds.

11.3.3 Procedures and Equipment

The adsorption of analytes from a water matrix requires several steps, including sorbent activation or conditioning, sample addition, washing, drying, and elution (20). Figure 11.8 shows the procedure schematically. In this case, the reversed-phase sorbent is first conditioned with hexane (the elution solvent) followed by methanol (the wash solvent) and water (the analyte solvent). The sample is applied slowly and the water is allowed to drain through the bed. The cartridge is then washed with methanol and finally eluted with hexane. Conditioning of the

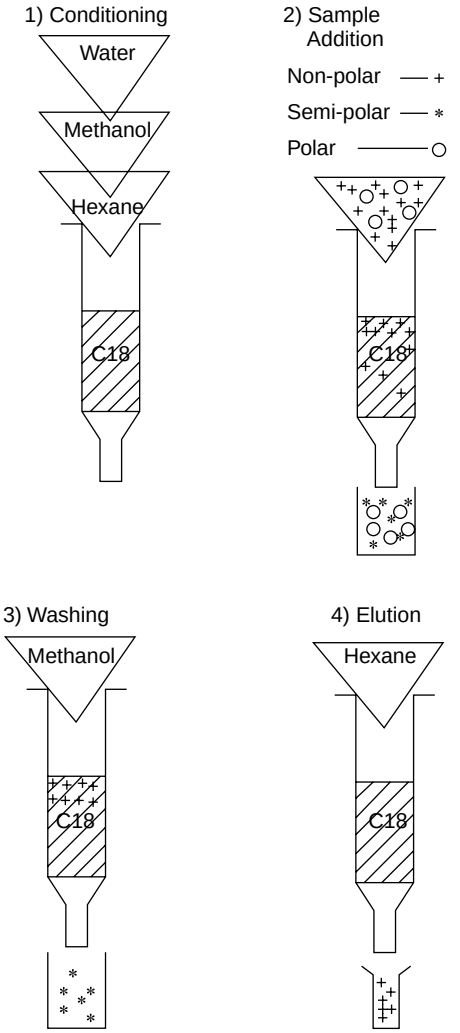

FIGURE 11.8 Steps involved in solid-phase extraction: (1) conditioning, (2) sample addition, (3) washing, and (4) elution.

sorbent ensures maximum interaction of the bonded silica sorbent with the analytes present in the liquid matrix. Typically 5–10 bed volumes of strong solvent or the elution solvent are passed through the sorbent bed by means of an aspirator; thus, for octadecylsilane (C_{18}) as the sorbent, hexane would be an appropriate solvent. In addition to sorbent activation, this will remove any residual contaminants that might be present on the sorbent. Typically the activation solvent is also the elution solvent. The activation solvent is then removed and replaced with an intermediate solvent, usually methanol. Finally, the bed is rinsed with water prior to the addition of sample. It is important that the bed not be overrinsed with water, or the bonded silica sorbent will no longer by wetted, resulting in low recoveries.

Sample addition can be accomplished by either pushing or pulling the liquid through the sorbent bed. One hundred percent recovery of the analytes may be possible without additional sample preparation. However, if necessary, several steps can be taken to improve the trapping efficiency of the analyte during sample addition. Changing the stationary phase will change the selectivity and may result in better extraction. In order to increase the adsorption efficiency of the solid sorbent, the analyte–water interactions must be weakened. This can be accomplished for nondissociating compounds by increasing the ionic strength of the aqueous matrix, thus increasing the partition ratio. This phenomenon is commonly called "salting out" and is accomplished by adding electrolytes such as sodium chloride and potassium sulfate to the aqueous matrix (13,14). For ionic analytes, a pH adjustment may be required to neutralize analytes. For complex samples, it may be necessary to increase the surface area of the sorbent available to the analytes. This increase can be accomplished by, either increasing the amount of sorbent (13) or decreasing the sample ratio. The addition of 1–5% methanol to the aqueous sample will keep the bed solvated and may improve analyte recoveries.

If necessary, the bed can be washed with a weak solvent to remove interfering contaminants. At least 20 bed volumes of wash solvent must be able to pass through the sorbent bed without eluting the analyte of interest. This step is often eliminated because it can result in a loss of analyte. Drying the sorbent can be accomplished in several ways. Water can be removed from the sorbent using positive or negative pressure (21), by a stream of nitrogen (20), by using a centrifuge (22), or by placing the sorbent in a desiccator for a period of time (23). It is very important to determine if this step will result in loss of analyte when the analyte is volatile (24). The analytes are eluted by a small volume of strong solvent. A general rule of thumb for solvent volume is elution of the analytes by 2 aliquots of strong solvent using 1 μL of solvent for every 1 mg of sorbent. However, several milliliters may be required to completely elute the analytes.

11.3.4 Applications of SPE

There are myriad applications of solid-phase extraction as a sample preparation technique for all types of chromatographic analysis. Perhaps the best way to

become informed about these is to peruse the applications guides provided by several of the vendors of solid-phase extraction equipment. As examples, on their Websites, Waters lists 113 application notes on SPE (25), Varian (26) lists several hundred, and J. T. Baker (27) also lists hundreds, along with an excellent introductory guidebook on SPE method development.

11.4 HEADSPACE EXTRACTION

Headspace extraction refers to a family of techniques, all of which involve partitioning equilibria between either liquid or solid phases and the vapor phase. In all of these techniques, an aliquot of the vapor phase is sampled and transferred to a gas chromatograph, usually through a transfer line to a classical inlet, such as split or splitless. In many of these techniques, sample concentration or peak focusing is required prior to chromatographic separation. The key error source is in ensuring that equilibrium is reached between the condensed and vapor phases prior to sampling. This is often difficult to determine, especially if the condensed phase is heterogeneous. Static headspace, in which the vapor above a sample is directly transferred to a gas chromatograph, is the most common technique, while dynamic headspace, in which the vapor phase moves through the condensed phase and is then passed over a sorbent to trap the analytes, is used for especially dilute samples. Headspace solid-phase microextraction, described in Section 11.5.1, has seen a tremendous rise in popularity since the early 1990s, while membrane-based headspace extractions, also described elsewhere in this chapter, are just beginning to see growth in use.

11.4.1 Basic Principles

Headspace gas chromatography (HSGC) has been available since the late 1960s (28) and is a rugged, robust, and popular method of sample preparation used for the introduction of volatile analytes to a gas chromatograph. The instrumentation for HSGC is both mature and reliable, and automated analysis with accurate control of all instrument parameters has become routine. There are two types of headspace analysis: static and dynamic. In *static* analysis, the analytes are sampled under conditions of equilibrium and in *dynamic*, the analytes are exhaustively extracted from the sample. Theses techniques are commonly called equilibrium headspace and purge and trap, respectively. For static HS, the sample is sealed in a vial where the analytes reach equilibrium between the gas phase (headspace) and sample (liquid or solid). Once at equilibrium the analytes are transferred to the chromatograph for analyses. In dynamic HS, equilibrium is never reached since the gases in the headspace are continuously removed from the vial. The volatile analytes are swept to a trap where they are held until analyses.

11.4.2 Static Headspace Extraction (SHE)

Static headspace extraction is typically used for analysis where complete extraction of the analytes is not required. The mechanism of analysis is straightforward:

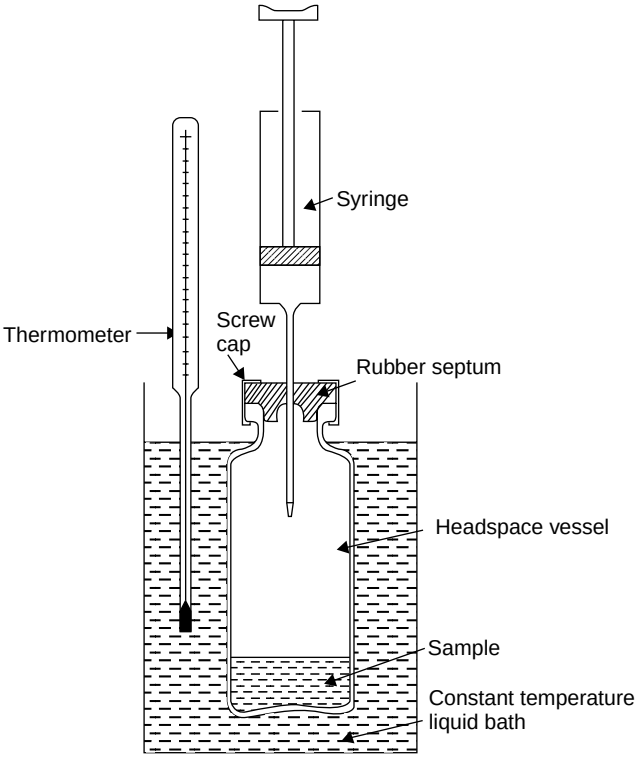

FIGURE 11.9 Diagram of a typical static headspace vial showing location of analytical sample and the vial headspace [reprinted with permission from H. Hachenberg, and A. P Schmidt, *Gas Chromatographic Headspace Analysis*, London, Heyden, (1977), p. 21].

a sample (solid or liquid), is placed in a headspace autosampler (HSAS) vial, typically 10 or 20 mL, and the volatile analytes diffuse into the headspace of the via. Figure 11.9 shows the basic principles of headspace sampling. Temperature control is critical in obtaining reproducible results. Once the concentration of the analyte in the headspace of the vial reaches equilibrium with the concentration in the sample matrix, a portion of the headspace is swept into a gas chromatograph for analysis. This can be done by either a manual gas tight syringe, as shown in Figure 11.9, or by use of an autosampler. For analysts that do not have access to an automated headspace sampler, the feasibility of the technique can be demonstrated by using manual gastight syringes. In addition, headspace solid-phase microextraction is an affordable alternative. This ease of initial sample preparation is one of the clear advantages of static headspace extraction. Often, for qualitative analysis, the sample can be placed directly into the headspace vial and analyzed with no additional preparation required. However, for quantitative analysis, it may be necessary to understand and optimize the matrix effects to achieve acceptable linearity, accuracy, and precision.

11.4.2.1 Instrumentation and Equipment for SHE

Figure 11.10 shows a typical schematic diagram for a HSGC instrumental setup. The analyte is introduced as a result of balanced pressure sampling, as demonstrated in Figure 11.11. In this example, in the standby position, the sample vial is brought to a constant temperature, above ambient, and in the pressurization position, the carrier gas is used to bring the vial to a constant pressure, greater than the column head pressure used for the separation. In sampling mode, the vial is then connected to the gas chromatographic column head through a heated transfer line, which is left open to the vial for a given period of time, and the sample is transferred to the column via a pressure drop from the vial to the chromatographic inlet pressure. Following transfer, the vial is again isolated. For automated systems, this sampling process can be repeated with the same vial or with the next vial.

11.4.2.2 Sample Preparation for SHE

In static headspace extraction, sample preparation for liquid samples is usually quite simple—most often the sample can just be transferred to the headspace sample vial and sealed immediately following collection of sample to minimize storage and handling losses (31).

Solids can be placed directly into a headspace vial and analyzed. However, for practical quantitative analysis of volatile compounds from solid particles, equilibrium between the headspace and the solid sample matrix must be reached in a sensible amount of time, less than 60 mins. Since analytes in large solid samples can be trapped in the interior of the sample, they may require excessive time to reach equilibrium or never reach it at all. Therefore, it is often necessary to change the physical state of the sample. Two common approaches are crushing or grinding the sample and dissolving or dispersing the solid into a liquid. The first approach increases the surface area available for the volatile analyte to partition into the headspace. The second approach transfers the sample to the liquid, which is preferred, since liquid or solution sample matrices are generally easier to work with. The analyte partitioning process into the headspace generally reaches equilibrium faster for liquids than solids. In addition, liquid equilibrium is well understood, unlike the unusual diffusion path problems, which often occur with

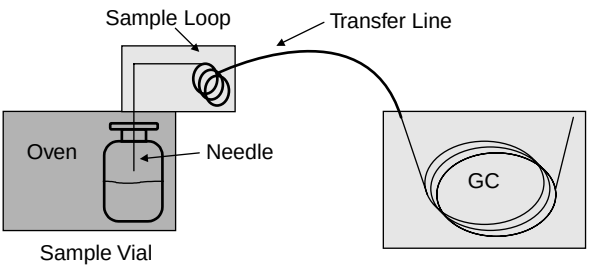

FIGURE 11.10 Schematic diagram of headspace extraction autosampler and GC instrument.

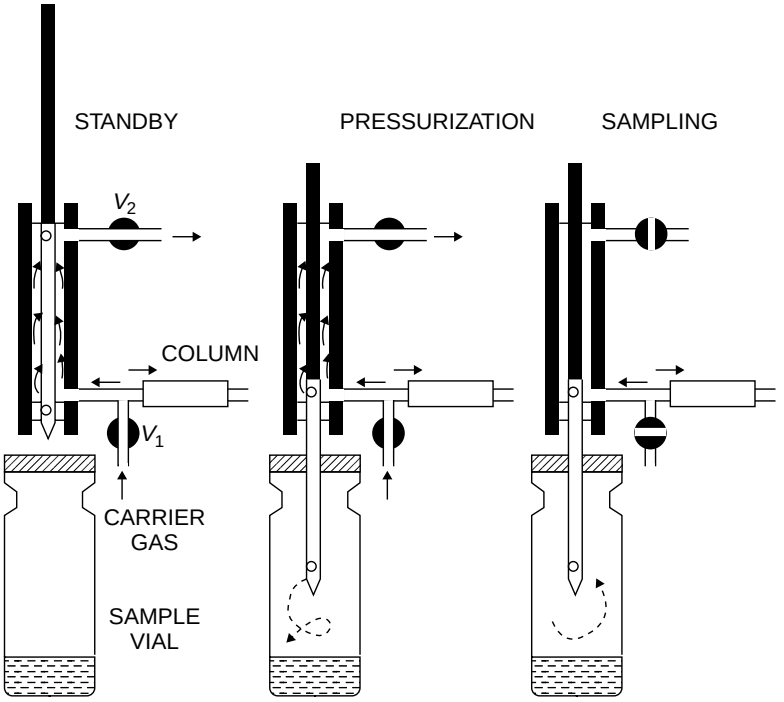

FIGURE 11.11 Steps for balanced pressure sampling in GC headspace analysis. Standby: the sample vial is temperature equilibrated at ambient pressure. Pressurization: the sample vial is pressurized to a pressure higher than the GC column head pressure and equilibrated. Sampling: the sample vial is opened to the transfer line and the GC inlet. The sampling time, temperature, and pressure drop determine the amount of sample transferred. (Reprinted with permission from B. Kolb and P. Popisil, in P. Sandra, ed., *Sample Introduction in Capillary Gas Chromatography*, Vol. 1, Huethig, Heidelberg, 1985.)

solids. One example of suspending or dissolving a solid in solution is seen in USP method <467>, which provides an approach for the analysis of methylene chloride in coated tablets. The sample preparation procedure calls for the disintegration of 1 g of tablets in 20 mL of organic free water via sonication. The solution is centrifuged after sonication and 2 mL of the supernatant solution is transferred to a HSAS vial and then analyzed by HSGC. (32)

11.4.2.3 Optimizing SHE Efficiency and Quantitation for Liquid Samples

Many factors are involved in optimizing static headspace extraction for extraction efficiency, sensitivity, quantitation, and reproducibility. These include vial and sample volume, temperature, pressure, and the form of the matrix itself, as described above. The appropriate choice of physical conditions may be both analyte- and matrix-dependent, and when there are multiple analytes, compromises may be necessary.

Ettre and Kolb showed that the analyte partition coefficient (K) and phase ratio (β) are the dominant factors for controlling headspace sensitivity (33)

$$A \approx C^{\mathrm{G}} = \frac{C^0}{K + \beta} \tag{11.23}$$

where A is the gas chromatographic peak area for the analyte, β is the phase volume ratio, C^{G} is the concentration of the analyte in the headspace, C^0 is the initial concentration of the analyte in the liquid sample, and K is the partition coefficient. The effect of the parameters K and β on static headspace extraction analysis sensitivity depends on the solubility of the analyte in the sample matrix. The equilibrium constant K is governed by the extraction temperature, and β is derived from the relative volume of the two phases. For volatile analytes that have a high partition coefficient (highly soluble), temperature will have a greater influence than the phase ratio. This is because the majority of the analyte stays in the liquid phase, and heating the vial drives the volatile into the headspace. For analytes with a low partition coefficient (less soluble), the opposite will be true. The volumes of sample and headspace have a greater influence on sensitivity than the temperature. Essentially, the majority of the volatile analyte is already in the headspace of the vial and there is little analyte left to drive out of the liquid matrix. This is illustrated in Figure 11.12, where a plot of detector response versus temperature for a headspace analysis shows that in an aqueous matrix increasing the temperature, increases the area counts for polar analytes, while the area for nonpolar analytes remains essentially the same (34).

The influence of analyte solubility in an aqueous matrix is also demonstrated in Figure 11.13, where the influence of sample volume is presented. For a polar analyte in an aqueous matrix (high K), the sample volume will have minimal effect on the area response, and for less polar analytes (low K), the sample volume has a noticeable effect on area response. The example presented in Figure 11.13 shows the effect of increasing the sample volume from 1 (a) to 5 (b) mL on area response for analytes cyclohexane and 1,4-dioxane (36). Salt may also be added to the samples to increase extraction recovery by the classical "salting out" effect. This effect is demonstrated in Figure 11.13b,c. Typically, sodium chloride is added to generate a salt concentration of >1 M. When examining Figure 11.13, one must remember that the concentration of the analytes has not changed, only the volume in the sample and the amount of salt added. Adding salt results in an increase in peak area of 1,4-dioxane (peak 2) and no change in cyclohexane (peak 1). Meanwhile, the result of changing sample volume is an increase in the area for cyclohexane (peak 1) and no change in 1,4-dioxane (peak 2). For an analyte with a large partition coefficient the impact of β is insignificant on the area. For example ethanol has a K value of $\sim$ 1000 (33). For example, a 10-mL headspace vial may be filled with 1 or 5 mL of the analyte solution $C_{\mathrm{G}} = C_{\mathrm{o}}/(1000 + 9)$ or $C_{\mathrm{G}} = C_{\mathrm{o}}/(1000 + 1)$, respectively. The difference in the results of these two calculations will be negligible. One can also see that for analytes where K is small the effect of β will be significant. This phenomenon is

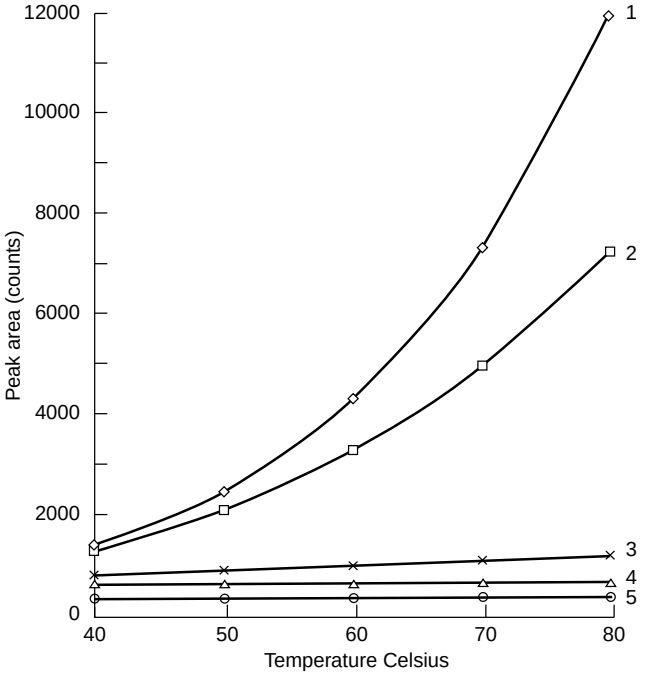

FIGURE 11.12 Influence of temperature on headspace sensitivity (peak area values, counts) as a function of the partition coefficient K from an aqueous solution with $\beta = 3.46$. The volatiles plotted above are ethanol (1), methyl ethyl ketone (2), toluene (3), *n*-hexane (4), and tetrachloroethylene (5). (Reprinted with permission from B. Kolb and L. S. Ettre, *Static Headspace-Gas Chromatography: Theory and Practice*, Wiley-VCH, New York, 1997, p. 26. Copyright 1997, John Wiley and Sons.)

extremely useful for the developmental chemist when method robustness is more important than sensitivity.

Choosing a matrix solvent that has a high affinity for the volatile analytes minimizes problems with sample and standard transfer from volumetric to the headspace vials. Also, if a second analysis of the headspace vial is necessary, the drop in signal from the first to the second injection will be negligible. To determine the impact of β when K values are not readily available, simply prepare the analytes in the desired matrix (aqueous or organic) and determine the area counts versus sample volume. Analytes with high K values will show no change in area counts relative to the volume of solution.

11.4.3 Quantitative Techniques in Static Headspace Gas Chromatography

The four most common approaches to quantitative HSGC calibration are classical external standard, internal standard, standard addition, and multiple-headspace

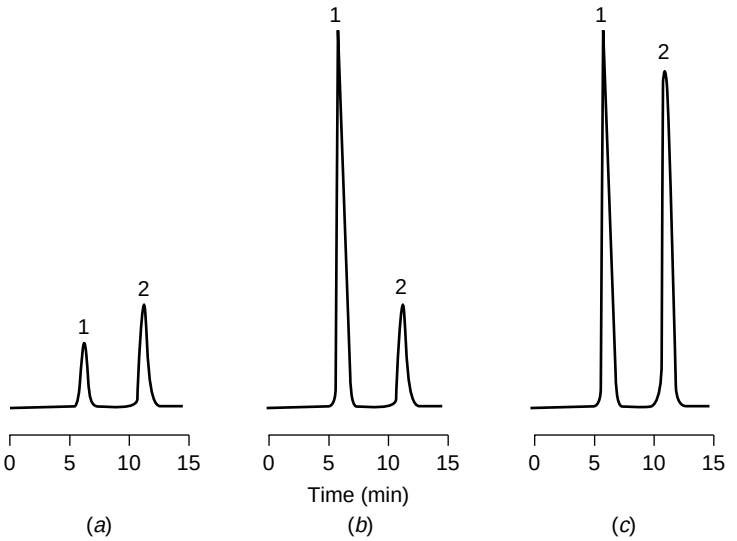

FIGURE 11.13 Analysis of three samples of an aqueous solution of cyclohexane (0.002 vol%) and 1,4-dioxane (0.1 vol %) in a 22.3-mL vial: (a) 1.0-mL solution ($\beta = 21.3$); (b) 5.0-mL solution ($\beta = 3.46$); (c) 5.0-mL solution ($\beta = 3.46$) to which 2 g NaCl was added. Headspace conditions: equilibration at 60°C, with shaker. Peaks: 1 = cyclohexane, $2 = 1,4-dioxane. (Reprinted with permission from B. Kolb and L. S. Ettre, *Static Headspace-Gas Chromatography: Theory and Practice.* Wiley-VCH, New York, 1997, p. 30. Copyright 1997, John Wiley and Sons.)

extraction (MHE). The choice of technique depends on the type of sample being analyzed.

11.4.3.1 External Standard Calibration

External standard quantitation involves the preparation of a classical calibration curve, as shown in Figure 11.14a. Standard samples are prepared at various concentrations over the desired range and analyzed. A calibration curve is then generated, with raw gas chromatographic peak area plotted against standard concentration. The concentration of each analyte is then determined from their respective peak areas. This method is best for analytes in liquid samples that have high K values where the matrix effects on the analyte response are minimal. If the analyte has a low solubility in the sample matrix, preparation of standards via serial dilution is possible. However, it may be important to match the standard and sample matrix. For both methods it is good practice to validate the recovery of the analytes against the standards by demonstrating equivalence in the response between the standards and samples. For example, when assaying pharmaceuticals the standards may be prepared in water and the sample may contain the analytes of interest and the remaining components of the pharmaceutical agent. If the matrix does not significantly effect the equilibrium of the analytes, acceptable recoveries will be achieved and matrix matching is not necessary. For

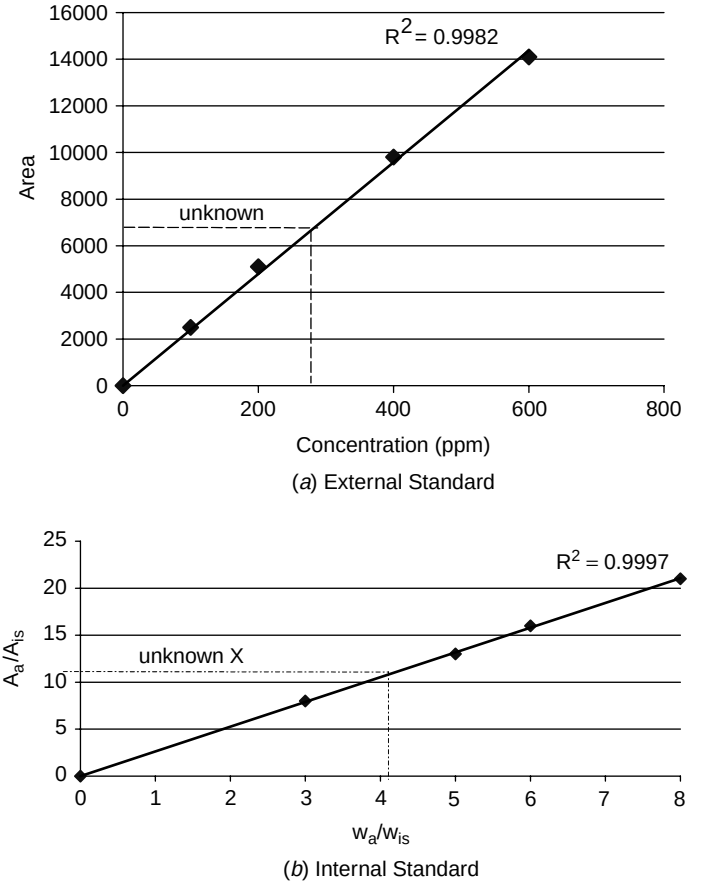

FIGURE 11.14 Calibration curves: (a) external standard; (b) internal standard.

solid samples, dissolving or dispersing in a liquid, demonstrating equivalence between standards and samples is preferred to matrix matching, since this simplifies standard preparation. Often this allows the analyst to prepare one set of samples for multiple assays where the analytes are identical and the matrices are similar but not identical. The main difficulty with external standard calibration is that is does not compensate for any variability in the chromatographic injection or sample preparation.

11.4.3.2 Internal Standard Calibration

Internal standard calibration can be used with headspace to compensate for variation in analyte recovery and absolute peak areas due to matrix effects, variability that results from sample preparation, and chromatographic injection variability. Prior to sample preparation, a known quantity of a known additional analyte is added to each sample and standard. Ideally, this compound, called an *internal*

standard, should not be present in the sample, be pure, and have chemical (for the extraction) and chromatographic properties similar to those of the analytes of interest. To prepare a calibration curve, shown in Figure 11.14b, the standards, which contain the internal standard, are chromatographed. The peak areas of the analyte and internal standard are recorded. The ratio of areas of analyte to internal standard is plotted against the concentrations of the known standards. For the analytes, this ratio is calculated and the actual analyte concentration is determined from the calibration graph.

11.4.3.3 Standard Addition Calibration

In standard addition calibration, an additional known quantity of the analyte is added directly to the samples, following an initial analysis. By adding one or more aliquots of standard, a calibration curve can be prepared. The concentration of analyte in the sample can then be determined by extrapolating the calibration curve, as shown in Figure 11.15. For this method, analyte response must be linear throughout the range of concentrations used in the calibration curve. A practical approach to standard addition is to divide up the sample into several equal portions then add increasing levels of standard. The samples are analyzed and area response versus the final concentration is plotted. The final concentration of the standard is the concentration of the standard after it is added to the sample. The original concentration is then determined by extrapolation to the x-axis (abscissa) (38,39). Alternatively, a single additional sample can be prepared and the original concentration of the analyte can be determined from the

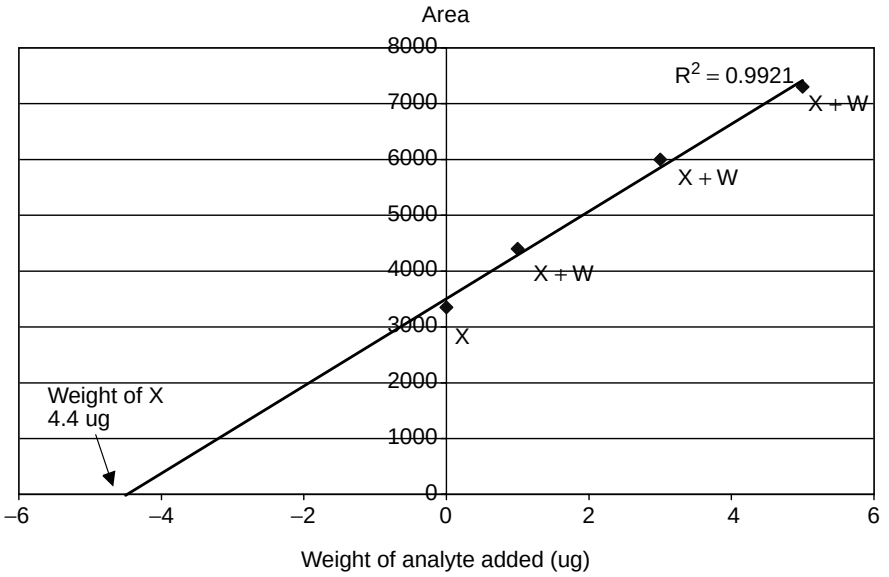

FIGURE 11.15 Standard addition calibration curve.

following equation:

$$\frac{\text{Original concentration of analyte}}{\text{Final concentration of analyte (sample + standard)}} = \frac{\text{area from original sample}}{\text{area from (sample + standard)}} \tag{11.24}$$

To calculate the original concentration of the sample using Equation 11.24, the final (diluted) concentration of the sample is expressed in terms of the initial concentration of the sample. Then the initial concentration of the sample is determined (40). It is important to remember that the sample and the standard are the same chemical compound.

11.4.4 Multiple-Headspace Extraction

Multiple-headspace extraction (MHE) is used to find the total peak area of an analyte in an exhaustive headspace extraction, which allows the analyst to determine the total amount of analyte present in the sample. The basic method involves successive analyses of the same sample vial. This technique, along with the mathematical models behind it, was originally presented by McAuliffe (41) and Suzuki et al. (42). Kolb and Ettre provide an in-depth presentation of the mathematics of MHE in their book (43), and the reader is encouraged to reference that work for further information on the mathematical model.

The advantage to MHE is that sample matrix effects (which are mainly an issue only with solid samples) are eliminated since the entire amount of analyte is examined. This examination is done by performing consecutive analyses on the same sample vial. With the removal of each sample aliquot from the vial, the partition coefficient K will remain constant; however, the total amount of analyte in the sample will decline with each analysis as the analyte migrates into the vial headspace. When the peak area eventually falls to zero, one knows that the amount of analyte in the sample has been completely exhausted.

The process described above is, however, not in common practice. MHE has been simplified through laboratory use, and in practice, a limited number of consecutive extractions—usually two to four (44)—are taken. Then a linear regression analysis is used to mathematically determine the total amount of analyte present in the sample.

11.4.5 Purge and Trap

For the analysis of trace quantities of analytes, or where an exhaustive extraction of the analytes is required, purge and trap, or dynamic headspace extraction methods, are preferred over static headspace extraction methods. Purge and trap has been used for both solid and liquid samples, which include environmental [water (45–47) and soil], biological (47,48), industrial, pharmaceutical, and agricultural samples. Like SHE, purge and trap relies on the volatility of the analytes

to achieve extraction and release from the matrix. However, the volatile analytes and matrix are not allowed to reach a state of equilibrium. This is accomplished by continually sweeping carrier gas across the headspace of the sample matrix, thus providing a continuous concentration gradient, which aids in the extraction of the analytes. Once in the carrier gas, the analytes are swept from the vial and trapped on a sorbent prior to analytical analysis.

Figure 11.16 shows a schematic representation of a typical purge-and-trap system. In this system, carrier gas (helium or nitrogen) is bubbled through the analytical sample. A six-port valve is used to pass the carrier gas over a sorbent trap. Purging is performed for a predetermined period of time, often until extraction is exhaustive. Following purging, the six-port valve is actuated and the carrier-gas (helium) flow is directed over the sorbent trap (which may be heated) to the chromatographic inlet, transferring the trapped analytes. Often, this must be combined with a cryogenic focusing trap at the GC column head to refocus the analyte bands.

There are several methods of analyte trapping: cryogenic, sorbent (50), and column focusing. Each of these methods has advantages and disadvantages. In general, the trap should do the following: retain the analytes of interest, not introduce impurities, and allow rapid injection of analytes to the column.

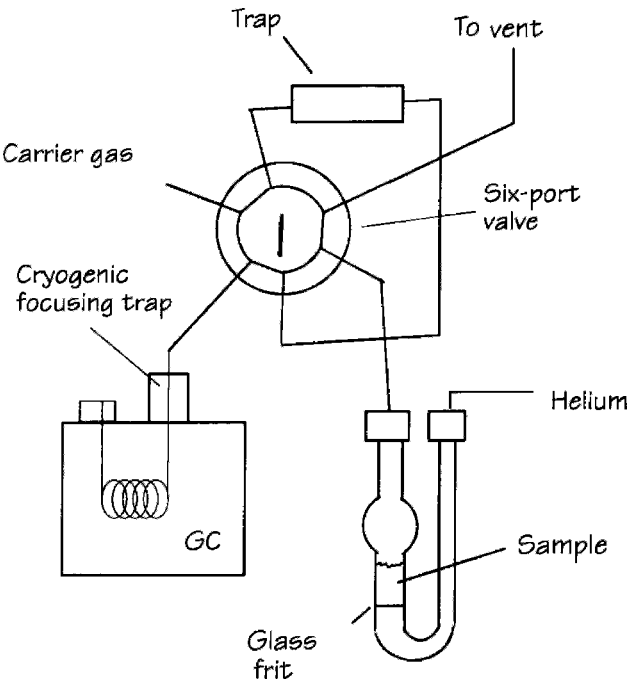

FIGURE 11.16 Schematic diagram of a typical purge-and-trap GC system (reprinted with permission from S. Mitra and B. Kebbekus, *Environmental Chemical Analysis*, Blackie Academic Press, London, 1998, p. 270).

11.5 SORBENT-BASED MICROEXTRACTIONS

The drive to completely eliminate organic solvents from analytical extractions led to the development of solid-phase microextraction (SPME) in the early 1990s (51). SPME operates in a fashion similar to liquid–liquid extraction, except that the organic liquid phase is replaced with a stationary-phase-coated fused-silica fiber. This fiber is contained within a syringe device that provides both protection and easy transport of the fiber phase. SPME has seen wide application in a tremendous variety of fields, leading to three texts (52–54) and an application guide containing hundreds of references, organized by application (55). Originally, SPME was performed by direct immersion in the analyte-containing aqueous solution. Headspace SPME was developed in 1993 (56), and an HPLC interface was demonstrated in 1995 (57).

The rapid rise in popularity of SPME had led to an increased interest in all types of sorbent-based extractions. Stirbar sorptive extraction (SBSE) is an outgrowth of SPME that employs a coated stirbar as the organic phase. Finally, there are a number of flowthrough extraction configurations that are combined with thermal desorption. These techniques offer interesting alternatives to classical solvent and headspace extractions.

11.5.1 Solid-Phase Microextraction (SPME)

Diagrams of several SPME devices are provided in Figure 11.17. These are configured for manual and automated sampling and for HPLC interfacing. There is also a version optimized for portable sampling in the field. When SPME is used for analysis, first the syringe needle is placed into the analyte solution or into the headspace and the coated fiber is exposed. Once the system is brought to equilibrium, the coated fiber is retracted into the syringe needle and removed from the sample vial. The needle is then transferred to a heated inlet, and the analytes are thermally desorbed into the gas chromatograph. There have been several variants on the basic SPME device reported, including an in-tube configuration, in which the extraction phase is placed within a capillary tube (58) and internally cooled (59) and heated (60) devices. The texts by Pawliszyn (52,53) provide details on the many possibilities.

The theory of SPME can be extended from the classical liquid–liquid extraction theory described above (61). Since in most SPME analyses, analytes are volatile and dissolved in an aqueous phase, a three-phase system, including the aqueous phase, the fiber phase, and the headspace phase above the aqueous, is described. The mass of analyte extracted into the fiber phase is given by

$$n = \frac{K_{12}V_2[\mathrm{A}]_1^0 V_1}{K_{12}V_2 + K_{13}V_3 + V_1} \tag{11.25}$$

where n is the mass of analyte extracted into the fiber phase and the subscripts 1, 2, and 3 refer to the fiber, sample, and headspace phases, respectively. If the

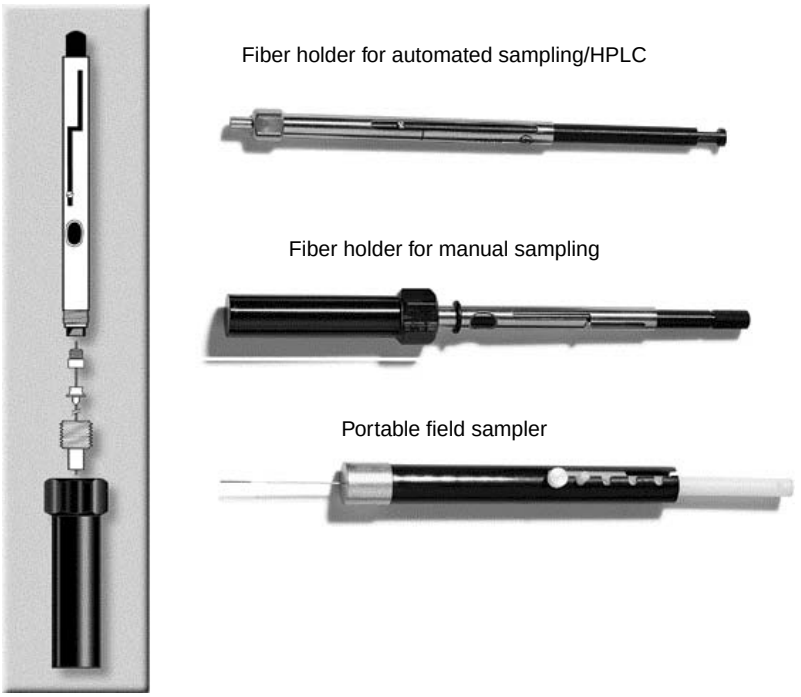

FIGURE 11.17 SPME fiber holder assemblies (courtesy of Supelco, Inc., Bellfonte, PA).

headspace volume is minimized, this equation is reduced to

$$n = \frac{K_{12}V_1V_2[\mathrm{A}]_1^0}{K_{12}V_2 + V_1} \tag{11.26}$$

and if it is assumed that the fiber phase volume is much smaller than the aqueous phase volume ($K_{12}V_2 \ll V_1$), typically occurring when the sample volume is very large (liters) then the equation further reduces to

$$n = K_{12}V_2[\mathrm{A}]_1^0 \tag{11.27}$$

Several useful conclusions about SPME, which, in a sense, may be described as LLE with the organic phase typically of much smaller volume than the aqueous phase, may be drawn from these equations:

1. The mass of analyte extracted into the fiber is independent of the fiber location, whether directly immersed in the aqueous phase or in the headspace above it, assuming that the system is fully brought to equilibrium, and as long as the phase volumes are constant.

2. The mass of analyte extracted into the fiber is independent of the aqueous phase volume, if the aqueous phase volume is very large.
3. Factors that affect the phase volumes and equilibrium constants, such as temperature, pressure, aqueous phase pH, additives, and matrix interferences, may strongly affect extraction efficiency and performance and will need to be controlled carefully. The practical consequences of salt and pH effects are shown in Figure 11.18.

In SPME, the kinetics of extraction must also be considered in determining the mass of analyte extracted and the extraction efficiency and are often the more difficult portion of the extraction to control. In his text, Pawliszyn (52) provides a detailed treatment of extraction kinetics kinetics in SPME, with experimental examples. The general conclusion is that the time required to reach equilibrium in SPME is driven by diffusion kinetics of the analyte in the phases that are

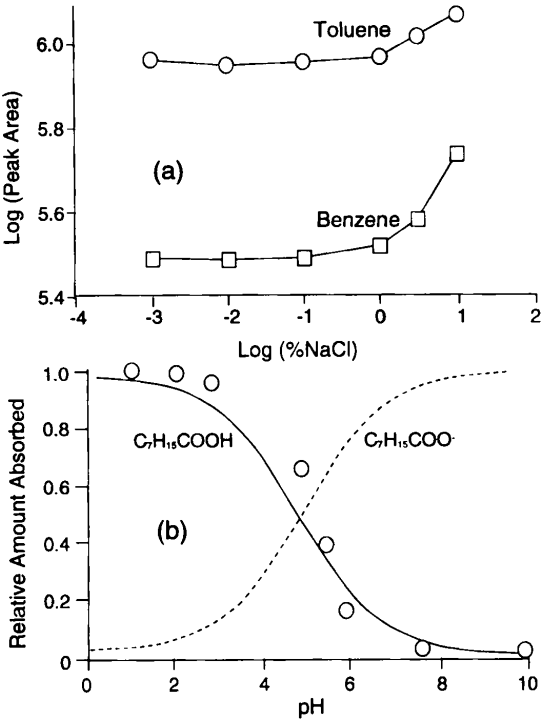

FIGURE 11.18 Effects of salt and pH on SPME extraction: (a) peak area versus salt concentration for benzene and toluene [reprinted with permission from C. L. Arthur, L. M. McKillam, K. D. Buchholz, and J. Pawliszyn, *Anal. Chem.* **64**, 1960 (1992), copyright 1992, American Chemical Society]; (b) amount absorbed versus pH for an ionizable compound [reprinted with permission from B. X. Yang and T. Peppard, *LC/GC* **13**, 882 (1995), copyright 1995, Advanstar Publications].

present. The important conclusions that affect SPME method development are summarized here:

1. If it is assumed that distribution constants remain constant with changes in concentration, then the sample concentration has no effect on the equilibration time.
2. Agitation conditions are the strongest factor in determining equilibration time for direct extraction from aqueous samples.
3. Thicker fiber coatings increase both the mass of material that can be extracted and the time required to reach equilibrium.
4. Equilibration time increases with larger distribution constant.
5. An increase in temperature will result in faster diffusion, therefore faster extraction, however, a decrease in the amount extracted may be seen, as equilibrium will more favor the headspace or aqueous phases. Optimizing and controlling temperature is therefore very important.

Thermal desorption into the gas chromatographic inlet is a third fundamental consideration in SPME. Most often, exposing the fiber phase in an inlet configured for splitless injection and desorbing the analytes under splitless conditions into a capillary column accomplishes this. Since there is no large volume of solvent vapor to accommodate, as in a classical splitless injection, a smaller-diameter glass inlet liner is often used with SPME. Kinetics and equilibrium are also involved with desorption, so there are a number of variables to optimize, including: fiber-coating materials and thickness, inlet liner diameter, carrier-gas type and flowrate, splitless time, and analytical column dimensions.

11.5.2 SPME Method Development

Although there are many variables involved, method development in SPME can be done systematically and efficiently. Figure 11.19 shows a flowchart for SPME method development. First, the basic extraction and instrumental parameters are chosen, followed by initial optimization of the extraction. Traditional method development procedures, including determination of linear range, detection limits, precision and accuracy, and validation, follow. As with any method development project, SPME method development begins with obtaining as much knowledge of the samples and purposes of the method as possible. If these are restricted to chemical and physical properties, they might include the sample matrix, the number and identity of analytes, the concentration range of the analytes, physical properties of the analytes, including melting and boiling points, pK_a, and chemical structure, and any information from the chemical literature about the extraction and chromatography of those or similar compounds.

11.5.2.1 Choosing Extraction Fibers and Chemistry

The variables involved in choosing extraction conditions include: the fiber coating and coating thickness, whether derivatizing is necessary, selection of an extraction mode and agitation method, automation options, and injection (desorption)

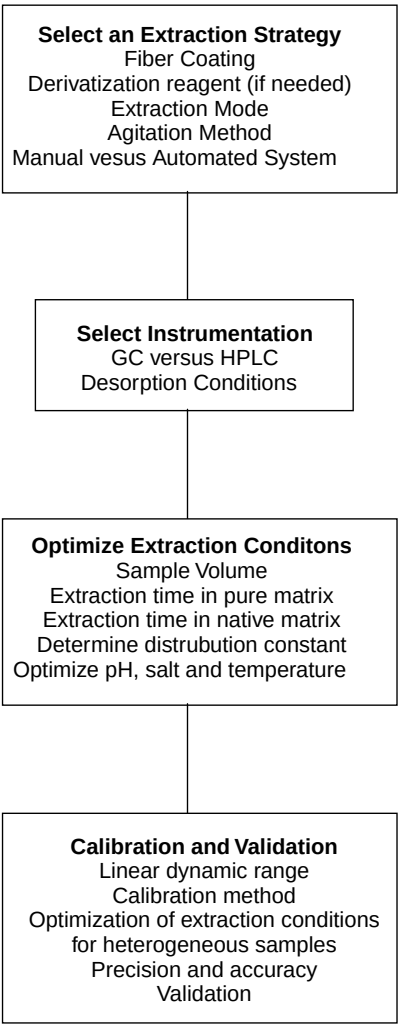

FIGURE 11.19 Flowchart for SPME method development (derived from J. Pawliszyn, *Solid Phase Micro-extraction Theory and Practice*, Wiley, New York, 1997, Table 4.1, p. 97).

options. Other than a few general comments, the optimization of gas chromatographic separation conditions is left to the discussions elsewhere in this text.

Table 11.2 lists the commercially available SPME fiber coatings, as of 2002, along with their most important analytes (62). In surveying the SPME literature, it is seen that approximately 80% of SPME methods have been developed using polydimethylsiloxane-coated fibers, so this is the coating of choice to begin and should be studied first, unless there are specific reasons to do otherwise. PDMS is especially useful because extraction behavior on PDMS-coated fibers can be

TABLE 11.2 Commercially Available SPME Fibers

Fiber	Film Thickness (μm)	Description	Application
Polydimethylsiloxane (PDMS)	100	Nonbonded	Most commonly used; nonpolar and semipolar volatile analytes
Polydimethylsiloxane (PDMS)	30	Nonbonded	Nonpolar and semipolar volatile and semivolatile analytes
Polydimethylsiloxane (PDMS)	7	Bonded	Less volatile non and semipolar analytes
Polydimethylsil-oxane/divinylbenzene (PDMS/DVB)	65	Partially crosslinked	Volatile compounds, amines, nitro aromatics
PDMS/DVB	60	Partially crosslinked	HPLC
Polyacrylate	85	Partially crosslinked	Moderately and highly polar analytes
Carboxen/PDMS	75	Partially crosslinked	Very volatile analytes
Carboxen/PDMS	95	Highly crosslinked	Very volatile analytes
Carboxen/DVB	50/30	Partially crosslinked	Odor compounds
Carbowax/DVB	70	Partially crosslinked	Polar compounds
Carboxen/templated resin	50	Partially crosslinked	HPLC
DVB/carboxen	50/30	Highly crosslinked	Flavors and volatile compounds

Source: Adapted from SPME fiber package insert, Supelco, Inc., Bellfonte, PA, 1999 and from Supelco Website (`www.supelco.com`).

predicted from retention behavior on PDMS-coated capillary columns, and these is a wealth of information on PDMS-coated columns (63,64). If another coating needs to be chosen (PDMS does not appear to offer the required extraction efficiency), then Figure 11.20 can be used as a rough selection guide. In general, the thinnest coating that can provide the needed extraction recovery should be used. Further, for polar analytes, the polyacrylate coating is best and volatile analytes can be extracted effectively using the mixed-phase coatings, based on either PDMS or Carbowax. If there are a number of analyte types, then compromises may be necessary, or a custom coating may need to be developed (65)

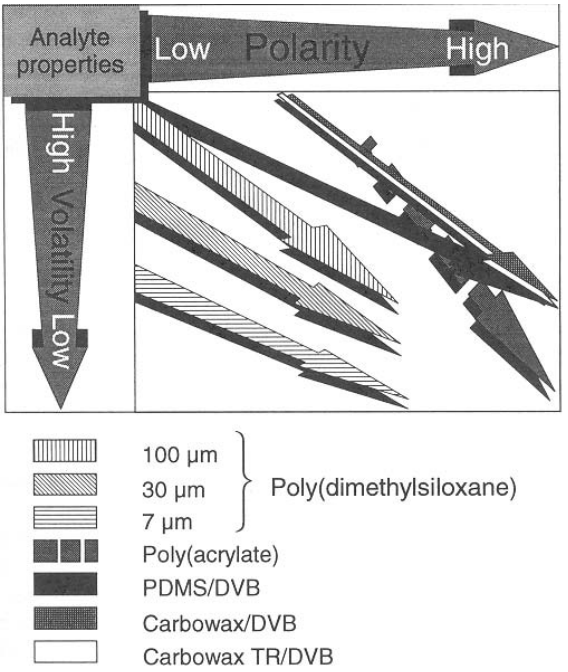

FIGURE 11.20 SPME fiber selection guide (reprinted with permission from J. Pawliszyn, *Solid Phase Micro-extraction Theory and Practice*, Wiley, New York, 1997, Figure 4.1, p. 99).

comparison of extraction conditions among several fibers is one of the basic and most important steps in SPME method development. Moeder et al. (66) provide a thorough discussion of fiber-coating selection for several biologically active substances, including endocrine disruptors and pharmaceuticals. Some of their extraction efficiency results are shown in Figure 11.21. They concluded that, for pharmaceuticals, the PDMS fibers showed the poorest result, and that while the polyacrylate fiber showed the overall best result, in some situations, especially with thermally labile analytes, the mixed PDMS divinylbenzene fiber proved best.

Where necessary, derivatization may be used in conjunction with SPME in the analysis of analytes that exhibit low volatility, poor chromatographic behavior, or poor detector sensitivity (67). Derivatization may be performed in one of three ways:

1. By adding the derivatization reagent(s) directly to the sample vials prior to extraction (68)
2. By doping the SPME fiber with derivatization reagent prior to extraction (69)
3. By exposing the SPME fiber to the derivatization reagent following extraction (70)

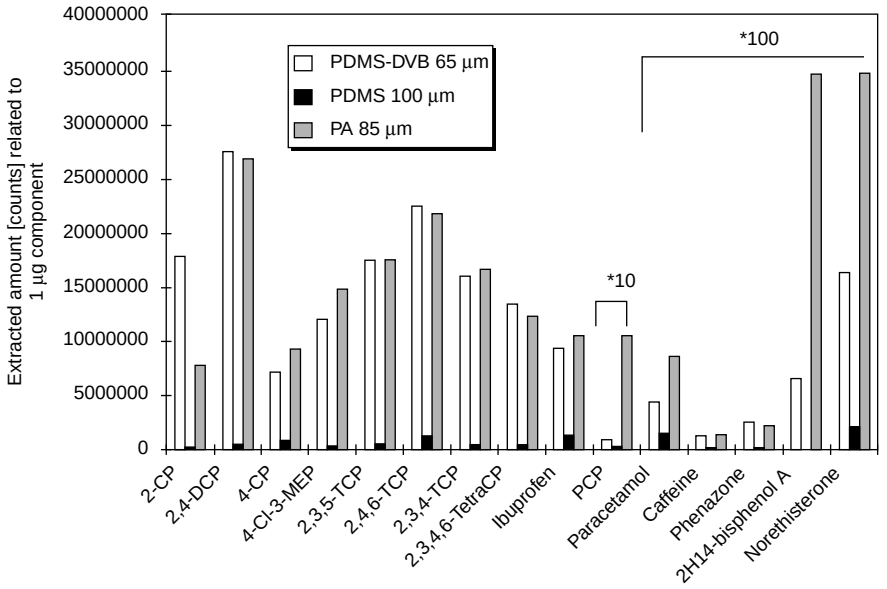

FIGURE 11.21 SPME extraction efficiency of pharmaceutical [reprinted with permission from M. Moeder et al., *J. Chromatogr A* **873**, 95–106 (2000); copyright 2000, Elsevier Science, Inc.].

Adding the derivatizing reagent directly to the sample vials can improve extraction efficiency by forming derivatization products that are more amenable to extraction. Typically, this might be done to make a polar analyte less polar, so that it could be more easily volatilized for headspace SPME. Fiber doping is perhaps the most generally useful of the derivatizing methods, as it can be readily applied in both headspace and direct-immersion extractions. The derivatizing reagent can be either sorbed or chemically bound to the fiber coating. It is interesting that the addition of derivatizing reagent to the fiber coating can have a significant effect on the equilibrium position of the extraction, often allowing exhaustive extraction. Finally, for analytes in complex matrices, postextraction derivatizing is the one technique that allows only extracted materials to be derivatized.

11.5.2.2 Extraction Mode and Agitation Method

The main issues in choosing an extraction mode and an agitation method involve the nature of the sample and matrix: volatility and affinity to the matrix. From the point of view of fiber lifetime, headspace extraction is preferred, but samples must be at least somewhat volatile and not very strongly bound to the matrix. For samples with lower volatility, direct immersion is preferred, although fiber lifetime is a consideration if the matrix is especially dirty. The second consideration in choosing the extraction mode is equilibrium versus exhaustive extraction. In SPME, equilibrium extraction is much simpler to perform, so it is generally

used. Exhaustive extraction is used when sample volume or concentration is very low, and in physicochemical studies.

Magnetic stirring is by far the most common agitation method. Again, this choice is determined in part by the choice of extraction mode. In headspace extractions, the sample is generally not agitated. In direct-immersion extraction, control of the agitation method plays a strong role in determining both equilibration time and extraction reproducibility. Agitation methods for SPME are summarized in Table 11.3. With magnetic stirring, caution must be observed that the fiber is placed off center, that the stirbar does not contact the fiber, and that its rotational speed is constant from run-to-run. Magnetic stirring is very difficult to use with automated systems, so techniques such as fiber movement are employed.

11.5.2.3 Optimization of Desorption Conditions

The majority of SPME applications have involved desorption into a gas chromatograph as the separation method. Most commonly, desorption is performed using a classical splitless inlet, with a narrow-bore glass liner. Under splitless conditions, with slow linear velocity through the glass liner, the desorption and transfer process in the inlet is slow, often requiring minutes, necessitating band focusing following the injection process. Langenfeld et al. (71) and Okeyo and Snow (72) have provided summaries of optimization methods for SPME using splitless inlets. Figure 11.22 shows the effect of inlet liner inside diameter on the splitless desorption of a homologous series of hydrocarbons. It is seen that there is little effect on the peak width of the later-eluting compounds, while there is a dramatic effect on the lower-molecular-weight analytes. The injected band may also be focused by using a very low initial column temperature, a retention

TABLE 11.3 Summary of Agitation Methods for SPME

Method	Advantages	Disadvantages
Static (no agitation)	Simple, performs well for gaseous phase	Limited to volatile analytes and headspace SPME
Magnetic stirring	Common equipment, good performance	Requires stirring bar in the vial
Intrusive stirring	Very good performance	Difficult to seal the sample
Vortex/moving vial	Good performance, no need for a stirring bar in the vial	Stress on needle and fiber
Fiber movement	Good performance, no need for a stirring bar in the vial	Stress on needle and fiber, limited to small volume
Flowthrough	Good agitation at rapid flows	Potential for cross-contamination, requires constant flows
Sonication	Very short extraction times	Noisy, heats the sample

Source: Reprinted with permission from J. Pawliszyn, *Solid Phase Micro-extraction Theory and Practice*, Wiley, New York, 1997, Table 4.3, p. 107.

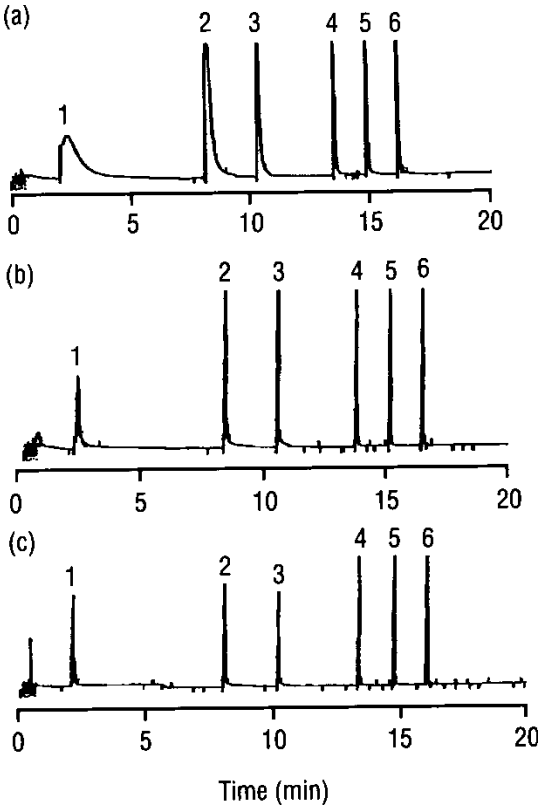

FIGURE 11.22 Effect of inlet liner diameter on SPME injection of hydrocarbons: (a) 4-mm-i.d. liner; (b) 2-mm-i.d. liner; (c) 0.75-mm-i.d. liner [reprinted with permission from P. Okeyo and N. H. Snow, *LC/GC* **15**, (1997) 1130–1136 (1997), Figure 3; copyright 1997, Advanstar Publications].

gap, a thick-film capillary column, or a combination of all three. Finally, desorption temperature is generally chosen by using the maximum recommended temperature for the fiber material and experimentally optimizing the desorption time, while considering possible analyte thermal degradation. Because they are less able to be sharpened by thermal focusing, the bands generated by volatile analytes will require more inlet optimization than those for less volatile analytes.

11.5.2.4 Optimization of Extraction Volume

In SPME, the sample volume is chosen on the basis of the analyte partition coefficient between the sample matrix and the fiber coating. Pawliszyn has described this in detail (73). The limiting sample volume can be estimated on the basis of the error of measurement E by

$$V_s = \frac{100 K_{fs} V_f}{E} \tag{11.28}$$

where V_s is the maximum volume of sample for maximum sensitivity, K_{fs} is the partition coefficient, V_f is the fiber coating volume, and E is the percent error of measurement. For a 100-μm-thick fiber coating and 5% allowable error, this indicates that a 2-mL sample volume is sufficient when K is less than 100, while for K up to 4000, a 40-mL sample volume is needed. In general, for larger values of K (which are likely to occur in more dilute samples), larger sample volumes are needed for maximum sensitivity. In headspace extraction, the volume of the gaseous phase should be minimized for highest sensitivity. The shape of the vial and the volume of the headspace also play roles in determining the amount and rate of extraction. In headspace SPME, since the amount of analyte extracted into the fiber is often much smaller than the amount in the headspace, the headspace capacity (the amount of analyte that is contained in the headspace) should be optimized and maintained much greater than the fiber capacity. This can be achieved most readily by increasing the headspace volume; however, typical sample vials are limited to about 40 mL, and this will result in a loss of sensitivity. Further, vial size and shape also play roles in determining extraction kinetics. If the headspace capacity is low, then the headspace volume and sample vapor contact areas play strong roles. For example, in static SPME, often used in headspace analyses or in automated systems, the cross-sectional area of the vial will determine the mass transfer rate between the vial and the headspace.

11.5.3 SPME Applications

Since its inception in 1990, there have been nearly 1000 papers published in the literature employing SPME. Headspace SPME alone cam account for must of the increase in publications on headspace-related techniques since 1997. Among these papers, there are myriad applications. Supelco has produced an applications guide that lists about 500 of these (74). In general, applications of SPME are seen in environmental analysis, including air, soil, and water, food, natural products, pharmaceuticals, and clinical and forensic analysis, plus numerous articles on theoretical aspects. SPME has proved to be one of the most versatile sample preparation techniques available.

11.5.4 Stirbar Sorptive Extraction (SBSE)

Perhaps the main drawback of SPME is that the fiber-coating volume is very small (less than 1 μL), limiting the mass of analyte that can be extracted. As an outgrowth of SPME, extraction using coated stirbars, which allow the use of much larger coating volumes, was developed by Baltussen et al. in 1999 (75). Basically, the extraction is performed using a phase coated stirbar that is exposed directly to the sample. A diagram of a coated stirbar is shown in Figure 11.23. As in SPME, when equilibrium is reached, the stirbar is removed from the sample and the analytes are thermally desorbed into the inlet of a gas chromatograph. The main difficult in this technique is that the gas chromatographic inlet must be a PTV inlet with thermal desorption capability, somewhat complicating the instrumentation. A diagram of SBSE desorption is also shown in Figure 11.23, showing the stirbar

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FIGURE 11.23 Instrumentation for SBSE: (a) stirbar is coated with polydimethylsiloxane; (b) stirbar is placed into a temperature-programmed GC inlet or thermal desorption apparatus for desorption of analytes and injection into the GC [reprinted with permission from J. Vercauteren et al., *Anal. Chem.* **73**, 1509–1514 (2001), Figures 1 and 2; copyright 2001, American Chemical Society].

placed inside the glass liner within a PTV inlet. An example application of SBSE involves the high-sensitivity determination of organotin compounds in various environmental samples (76). Organitin compounds are of interest as they are widely used in marine applications to extend the lifetimes of paints and as fungicides, yet they are highly toxic to marine animals and, although usage has decreased, they are still of concern as a marine pollutant. Chromatograms showing analysis of picogram levels of organotin compounds in harbor water extracted by SBSE and analyzed by GCICP/MS are shown in Figure 11.24. The exact chromatographic and extraction conditions may be found in the referenced article, but it is seen that the extraction required about 15 mins, followed by an

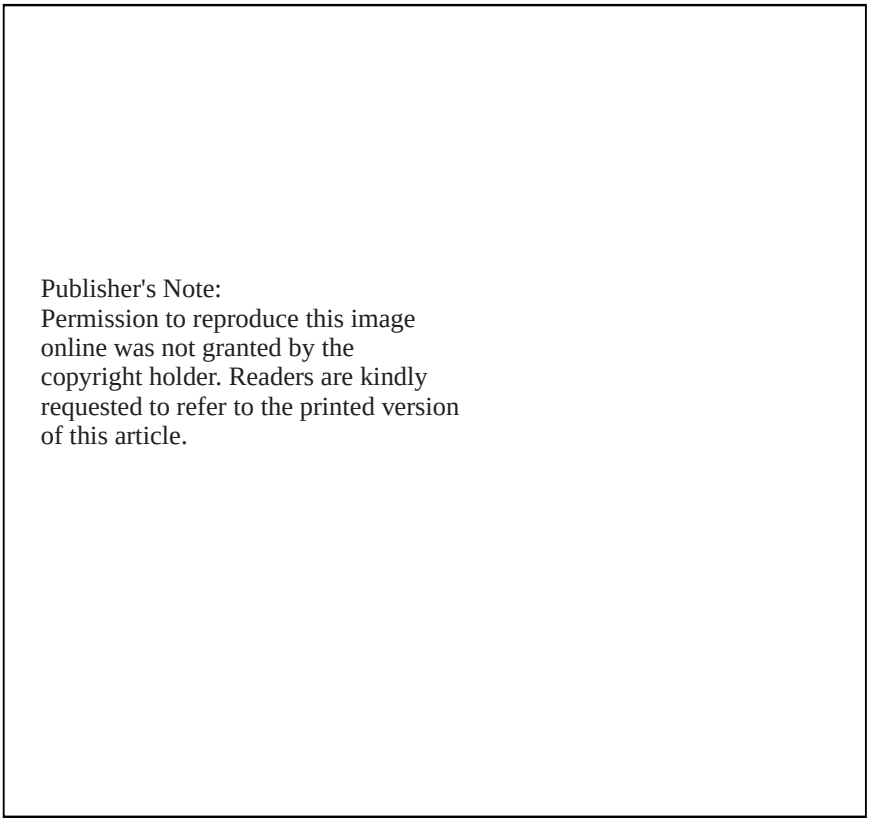
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FIGURE 11.24 Chromatograms of harbor water samples analyzed using SBSE/GCICP/MS: (a) tributyl tin (TbuT) was analyzed and triphenyl tin (TPhT) was internal standard (50 pg); (b) triphenyl tin was analyzed, and tricyclohexyl tin (TCT) was the internal standard (50 pg) [reprinted with permission from J. Vercauteren et al., *Anal. Chem.* **73**, 1509–1514 (2001), Figure 4; copyright 2001, American Chemical Society].

8-min gas chromatographic run. They found detection limits of about 100 fg/L (Femtograms per liter), with a linear dynamic range between 0.025 and 100 ng/L. Reproducibility was about ±12% for 10 consecutive extractions using the same stirbar. A wealth if additional information on SBSE can be found on the Website of its only vendor (77). SBSE presents an interesting alternative to traditional SPME, especially in cases where maximum sensitivity is needed.

11.5.5 Flowthrough Techniques

SPME and SBSE are both essentially static techniques, performed within the confines of a sample vial. One way to maximize extraction sensitivity as seen

in both traditional liquid- and vapor-phase extractions is to use a continuous extraction, such as Soxhlet extraction for liquid–solid extraction or purge and trap for vapor–liquid extractions. Continuous extractions using sorbent trapping can also be performed using flowthrough techniques. Several workers developed open tubular trapping, in which a liquid or gas sample is passed through a capillary that is coated with a stationary phase (78,79). To maximize efficiency, sampling is usually performed until one of the analytes is no longer fully retained, indicating that the full capacity of the stationary phase has been reached. Following this, the capillary can be desorbed either by heating or by extraction by an organic solvent, followed by injection into a gas chromatograph. A flowthrough technique introduced in 1997 involves the packing of polydimethylsiloxane particles into a glass tube, which can be transferred directly into a thermal desportion system or into a GC inlet (80,81). The packed bed contains about 300 μL of PDMS, providing even greater capacity and sensitivity. A flowthrough system for SPME has also been developed (82). A schematic diagram of an online flowthrough system for SPME is shown in Figure 11.25. This instrumentation is very similar to that used in the other flowthrough systems.

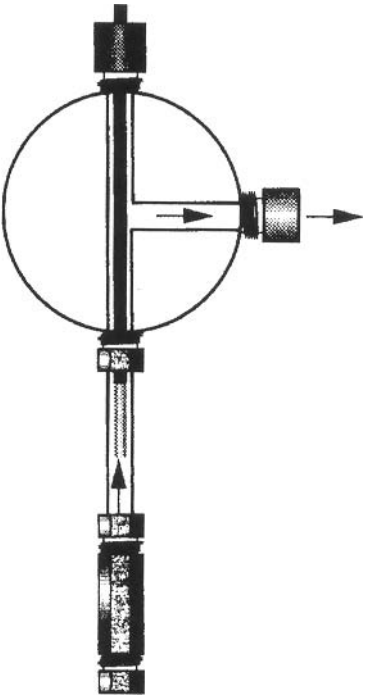

FIGURE 11.25 Flowthrough SPME extraction diagram; the fiber is contained within a tube through which the analyte solution and later, a desorption solvent, flows [reprinted with permission from R. Eisert and J. Pawliszyn, *J. Chromatogr. A* **776**, 293 (1997), Figure 1d; copyright 1997, Elsevier Science B.V.].

11.6 OTHER SAMPLE PREPARATION METHODS

While liquid–liquid, headspace, and sorbent-based extractions are perhaps the most commonly used and published sample preparation techniques for GC, there are numerous additional techniques to consider. While we do not attempt to fully describe every technique that has ever been used, the techniques described below are certainly of importance in the arsenal of sample preparation techniques for GC. These include supercritical-fluid extraction, accelerated solvent extraction, microwave-assisted extraction, pyrolysis, thermal desorption, and membrane-based extractions, plus comments on automation and derivatization.

11.6.1 Supercritical-Fluid Extraction

11.6.1.1 Fundamentals of Supercritical Fluids

Supercritical fluids (SFs) are dense gases above their critical temperature and pressure, possessing gaslike viscosities and diffusivities, and having densities and solvating properties that approach those of a liquid (83). Figure 11.26 is a typical phase diagram representing the three different phases of a pure compound, with the shaded area representing the supercritical fluid region. Above the critical temperature, an increase in pressure will not drive the fluid into the liquid phase (84).

The properties of SFs make them ideal for extracting analytes from solid matrices such as soils, agricultural products, foods, and solid sorbents. Supercritical fluids have the ability to maximize the extraction selectivity by controlling the temperature and pressure of the supercritical fluid (Figure 11.27) (85). Initially, the solubility of an analyte in a supercritical gas is dependent on solute vapor pressure; thus the solubility of the analyte in the gas first decreases with a rise in pressure reaching a point of minimum solubility. As the gas is compressed into the critical phase, there is a rapid increase in analyte solubility, which ends at a maximum pressure that is determined by the extraction temperature. Any additional increase in pressure will only slightly increase analyte solubility. Also, in

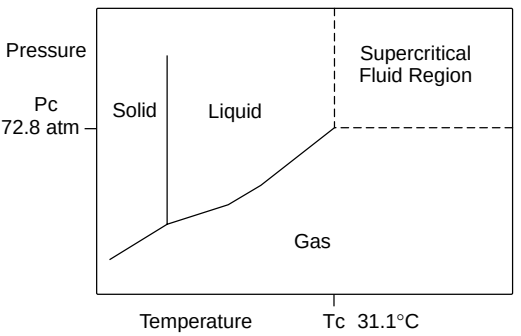

FIGURE 11.26 Phase diagram for carbon dioxide (Pc = critical pressure; Tc = critical temperature; Cp = critical point).

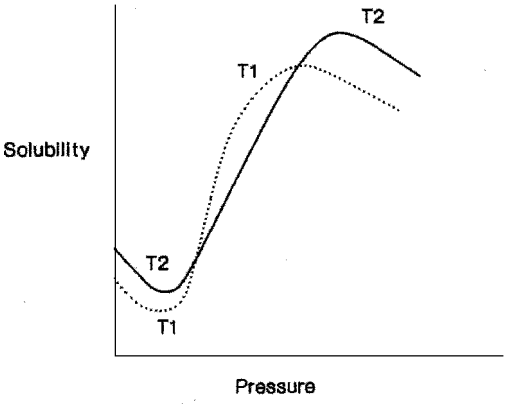

FIGURE 11.27 Generalized solubility isotherm as a function of pressure at two different temperatures; temperature 1 (T1) is greater than temperature 2 (T2).

some cases a higher extraction temperature will result in an increase in analyte solubility (86).

At least two factors play a role in the extractability of an analyte from a solid matrix by SFE: (1) the analyte must by soluble in the supercritical fluid and (2) the analyte solvent interactions must be more energetically favorable than those of the analyte and the matrix. To determine whether the analyte is soluble in the SF, knowledge of the physical properties of the analyte is helpful. The melting point of the solid can be vital, since analytes tend to be more soluble in SFs in their liquid states. Above the melting point, the mass transfer of the analyte into the SF is improved along with analyte solubility because the cohesive forces of the liquid are less than those of the solid. In addition, the vapor pressure can play a role in the solubility of an analyte, especially for multicomponent systems (87). Information on analysis of the analytes by supercritical-fluid chromatography may be helpful in determining the analyte solubility in a supercritical fluid (88).

If the analyte is soluble in a SF yet cannot be extracted from the matrix, the analyte matrix interactions may be too strong. The problem may be overcome by the addition of modifiers to the supercritical fluid, or by the direct addition of a modifier to the extraction vessel. Several papers dealing with the use of modifiers for supercritical-fluid chromatography (SFC) (89–91) and SFE have been published (92–94). Modifiers have two basic effects on the SFE of analytes from a matrix. They can interact with the surface of the matrix displacing the analyte into the SF. To distinguish between the two types of modifiers, they are commonly termed solvent modifiers and matrix modifiers, respectively.

11.6.1.2 Instrumentation for SFE

There are two basic modes of supercritical fluid extraction: static and dynamic. Both will be discussed in the following sections. The basic instrumentation required for both modes of SFE is similar. Figure 11.28 illustrates the minimum

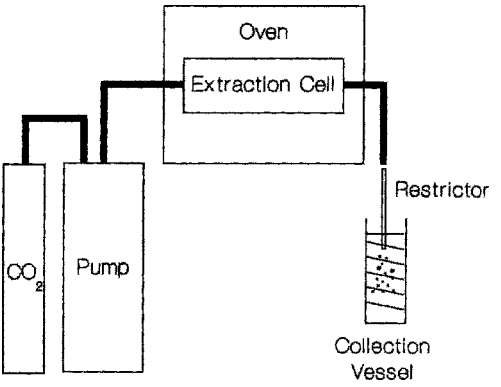

FIGURE 11.28 Instrumentation for SFE.

hardware required to perform a supercritical fluid extraction. The components of a system are as follows: (1) a source of high-purity fluid with an attainable critical temperature and pressure, (2) a high-pressure delivery system, (3) an oven, (4) an extraction vessel, (5) a restrictor, and (6) a sample collector.

Several fluids have been used as supercritical solvents; Table 11.4 shows the physical properties of several common SFs (95). The most common solvent is carbon dioxide because it has critical values that are easy to obtain (96), is nontoxic, becomes a gas at ambient temperatures and pressures, is inexpensive, and is mutually soluble with many organic compounds. Carbon dioxide can be obtained as a liquid from cylinders equipped with a dip tube. The cylinder headspace is often pressurized with 1500 psi of helium, which conveniently allows the liquid to be transferred to a delivery system.

The delivery system can either be a syringe pump, reciprocating piston pump, or gas compressor. Both syringe and reciprocating piston pumps are available on analytical-scale commercial instrumentation. These are used for analytical-scale extractions, while gas compressors are typically used for large-scale

TABLE 11.4 Physical Properties of Compounds Commonly Used as Supercritical Fluids

Fluid	Critical Temperature (°C)	Critical Pressure (atm)	Dipole Moment (D)
Carbon dioxide	31.3	72.9	0
Nitrous oxide	36.5	72.5	0.51
Ammonia	132.5	112.5	1.65
Pentane	196.5	33.3	0
Sulfur hexafluoride	45.5	37.1	0
Freon	111.8	40.7	0.17
Xenon	16.6	58.4	0

extractions (97). The pumps should be able to deliver pressures of $\leq$10,000 psi. To attain critical temperatures, the extraction cell is placed inside an oven. The oven can be a commercial SFE oven, gas chromatographic oven, or a home-built heating device. The sample matrix is usually placed inside a stainless-steel extraction vessel. Extraction vessels are available in a variety of shapes and sizes and are typically made of stainless steel able to withstand pressures of 350–680 atm. However, the use of non-stainless-steel composite is becoming more common. The later vessels allow for the use of disposable cells, which reduces the potential for cross contamination. Typical quantitative SFE samples are less than 10 g (98). Ideally, the extraction cell should be large enough to hold the sample, yet leave little dead volume in the extraction vessel. Any void volume in the extraction cell will increase the time required to flush the analytes from the vessel.

The pressure in the system is maintained by a restrictor after the extraction vessel. Historically, typical restrictors include (1) a tapered restrictor, typically a 10-cm $\times$ 50-μm-i.d. piece of fused-silica capillary that has been drawn at the end to an internal diameter of 5–7 μm; (2) a linear restrictor, typically 5–15 cm in length, 10–30 μm-i.d. piece of fused silica depending on the flowrate desired; (3) an integral restrictor, a piece of fused silica for which the end is melted into a ball and then filed down until the proper diameter is reached; and (4) a frit restrictor, a piece of fused silica that has the end plugged with porous silica wool (99). Today, commercial backpressure regulators are commercially available and an integral part of the instrumentation. After the analytes are extracted, they must by trapped in order to perform the appropriate analyses. Several trapping techniques are currently employed. These techniques include solvent trapping (100,101) (Figure 11.29), solid sorbent traps (92) (Figure 11.30), and direct online trapping (99).

11.6.1.3 Dynamic versus Static SFE

Like HSGC, SFE has two modes of extraction: dynamic and static (102). In the *dynamic* mode, the sample matrix is continuously flushed with fresh supercritical fluids, which pass through the sample matrix, solvate the analytes, and carry them to a trap where the analytes are collected.

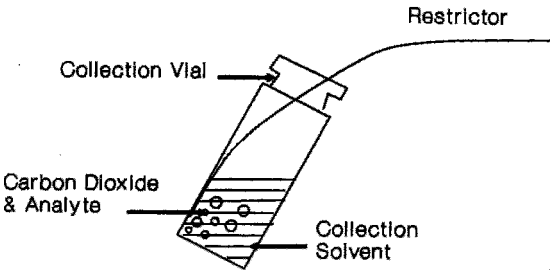

FIGURE 11.29 Solvent trapping in SFE.

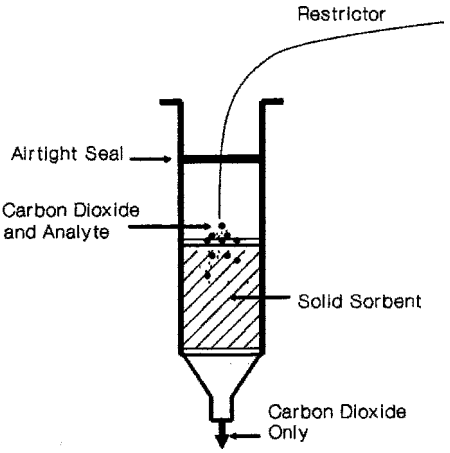

FIGURE 11.30 Trapping on a solid sorbent.

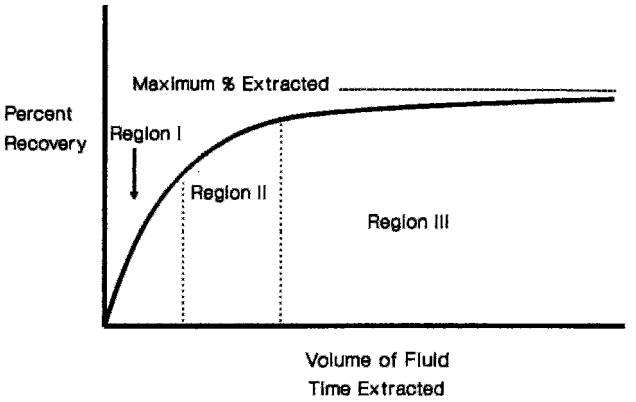

FIGURE 11.31 Extraction recovery versus fluid volume (static extraction) or extraction time (dynamic extraction).

The extraction profile for a typical dynamic extraction is depicted in Figure 11.31 (85). An extraction is divided into three different regions. In region I, the equilibrium controlled phase, the analytes are rapidly extracted from the matrix with the rate of extraction depending on the solubility of the analytes in the SF, the rate at which the SF passes through the extraction vessel, and the void volume of the extraction cell. Region I is the linear portion of the curve. Region II is the transition phase, where the extraction starts to become diffusion-limited because the analyte on the surface of the matrix has been swept out of the vessel. The result is a decrease in the rate of extraction as the rate becomes diffusion-limited. Finally, in region III, the diffusion-controlled phase of the extraction, the extraction rate is controlled by the diffusion rate and mobility

of the analyte within the sample matrix along with its desorption rate from the surface of the matrix.

In the *static* mode, the sample is placed into an extraction vessel, filled with a supercritical fluid at the appropriated temperature and pressure, and allowed to stand for a period. When the extraction is complete, the supercritical fluid is released through a trap to collect the analytes. Static extraction allows analytes with slow mass transfer time to be solvated by the SF. In addition, the use of a known concentration of modifier is possible by direct addition of the modifier to the extraction cell. The main limitation of static extraction is its inability to perform an exhaustive extraction. As in static headspace GC, and the traditional liquid–liquid extraction, as a result of the equilibrium of the analyte between the matrix and SF, one extraction can not exhaustively extract the analyte from the matrix. Consequently, it is often necessary to perform multiple static extraction. The use of SFE has been decreasing over the years in part due to the growth of accelerated solvent extraction (ASE), which employs much of the same instrumentation and methodology of SFE.

11.6.2 Accelerated Solvent Extraction (ASE)

This technique is very similar to SFE in that the analytes are extracted from the sample using a heated organic solvent at relatively high pressures. The solvents are typically the ones that were commonly used as additives in SFE. However, the solvents are not under supercritical-fluid conditions. This method of sample extraction is rapid and automated, which is useful to labs that perform numerous analyses of solid samples. As seen in Figure 11.32, ASE instrumentation is

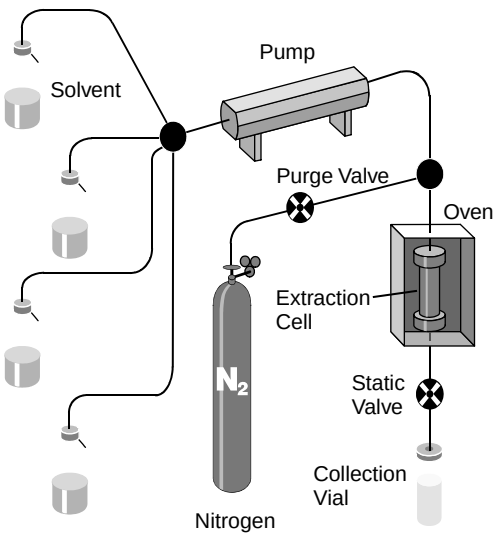

FIGURE 11.32 Schematic of an accelerated solvent extraction system (courtesy of Dionex Corp., Salt Lake City Technical Center.).

very similar to that used for SFE, a high-pressure pump is used to deliver the extraction fluid to a vessel that can with stand high pressures and temperatures. The vessel is heated and has an automated sealing mechanism, which controls the pressure and solvent flowrate, and the extract is collected in collection vessels. ASE has the advantage of being able to use traditionally employed organic solvents to elute analytes from a sample matrix. By heating the solvents to a higher temperature, the extraction efficiency is improved. The elevated temperatures increase the solvents extraction efficiency, which accelerates the rate of extraction and reduces solvent volumes and elution times (103). The operation of the instrumentation and the extraction process is also similar to that of SFE. The sample vessel is loaded, then placed in the ASE instrument where the extraction process is automated. The solvent is introduced to the vessel where it is heated and pressurized. The system is allowed to stand (static extraction), and after a period of time, a nitrogen purge of the vessel flushes the solvent to a sealed vial. These parameters can be optimized to influence the solubility, mass transfer, and desorption of the analytes from the sample matrix (104). Since the extraction solvents are organic, they are compatible with gas chromatographic analysis. A review of the literature shows that there have been approximately 150 publications on ASE since the late 1990s. The areas cover the extraction of environmental samples (105,106), natural products (107,108), foods (109,110), sediments (111,112), pharmaceuticals, and atmospheric particulate matter (113).

11.6.3 Microwave-Assisted Extraction (MAE)

The use of microwaves to heat the sample allows for rapid heating and extraction at above ambient temperatures and pressures. There are two approaches to MAE, use of a microwave-absorbing extraction solvent or a non-microwave-absorbing solvent. When using a microwave-absorbing sample, the sample and solvent are placed in a microwave compatible container, which can be sealed and capable to withstand the pressures that are generated during the extraction. The pressure generated is typically no more than a few hundred psi. Common materials used for MAE are composites, PTFE and quartz. The material must be able to withstand the increase in temperatures and be inert to the chemicals used in the extraction (114). Instrumentation for closed-vessel MAE is shown in Figure 11.33. The extraction can be optimized by controlling the heating time, method of heating—pulsed or continuous, vessel configuration—open or closed, agitating or stirring of the sample and rate of cooling. Microwave extraction has been studied and used for the extraction of a wide range of sample, such as cockroaches, black pepper, natural products (115), and extraction of lipids from fish (116). In addition to the extraction of analytes, MAE has also been used to determine the solubility of wood in hot water (117).

11.6.4 Membrane-Based Extractions

In the 1980s, interest in the use of nonporous membranes for sample enrichment arose as alternatives to solid-phase or liquid–liquid extractions. Several techniques were developed, including supported liquid membrane extraction (SLME)

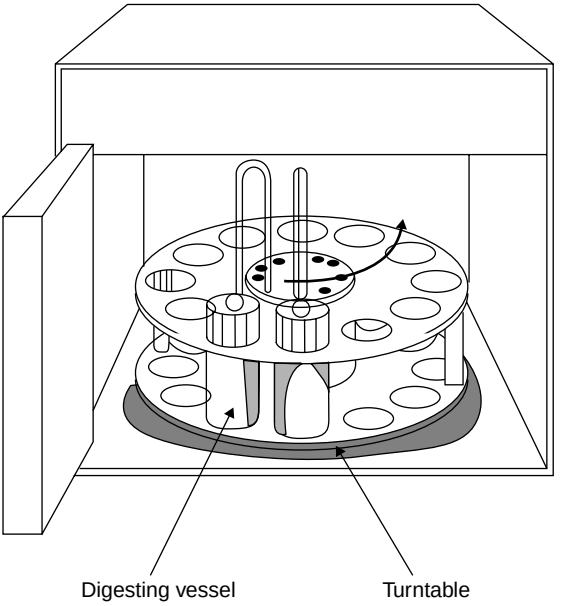

FIGURE 11.33 Closed-vessel microwave-assisted extraction system (reprinted with permission from H. M. Kingston and L. B. Jassie, eds., *Introduction to Microwave Sample Preparation*, American Chemical Society, 1998).

(118), microporous membrane liquid–liquid extraction (MMLLE) (119), polymeric membrane extraction (PME) (120), and membrane extraction with a sorbent interface (MESI) (121). Membrane extraction devices usually consist of a holder made from two blocks of inert material in which a groove has been machined. The membrane is placed in the groove and the blocks are clamped together to allow fluid to pass on either side of the membrane, or both inside and outside of the membrane in the case of a hollow fiber membrane. MESI, shown schematically in Figure 11.34, has proved to be the most natural of the membrane extraction techniques for interfacing with capillary GC, although there are examples in the literature from other techniques. In MESI, the acceptor phase is a gas and the effluent from the fiber is trapped on a sorbent, then thermally desorbed into the gas chromatograph. Membrane extraction techniques have recently been thoroughly reviewed (122).

11.6.5 Pyrolysis

Pyrolysis, the breaking apart of larger molecules into smaller ones using only thermal energy (123), is commonly interfaced to gas chromatography for the analysis of large molecules by thermal degradation. For example, polymers may undergo *chain scission*, in which the backbone of the polymer is broken up, resulting in smaller oligomers; *sidegroup scission*, in which the sidegroups are broken off; and *unzipping*, in which the polymer reverts almost entirely to monomer.

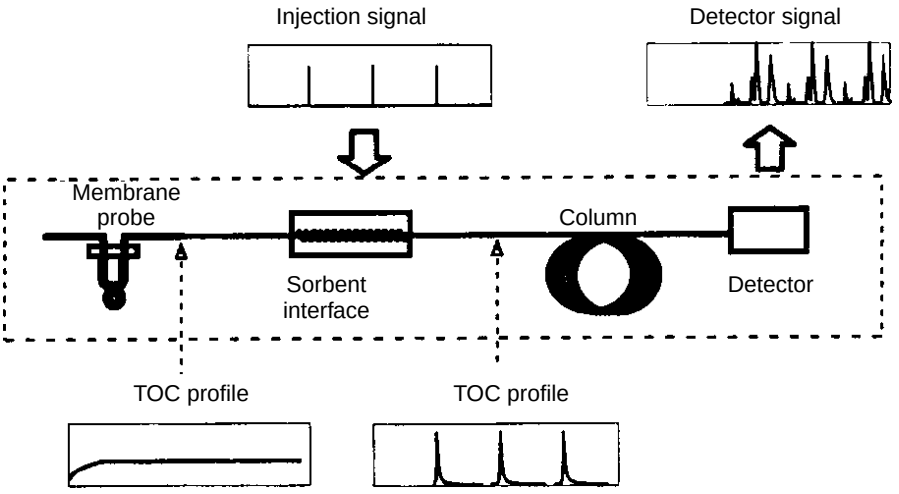

FIGURE 11.34 Schematic diagram of membrane extraction with a sorbent interface with a gas chromatograph (reprinted with permission from J. Pawliszyn, *Solid Phase Micro-extraction Theory and Practice*, Wiley, New York, 1997, Figure 1.3, p. 6).

The pyrolysis conditions must be adjusted so that these processes can occur rapidly enough to be analytically useful. Generally, this is done by increasing the temperature, with pyrolyzers capable of heating to 1400°C common. Typical temperatures are on the order of 500–800°C. A diagram of the connection of a typical pyrolyzer to a gas chromatograph is shown in Figure 11.35. The carrier gas is routed through an eight-port valve to pass it through the pyrolysis chamber, which sits directly on top of the inlet. The eight-port valve allows the pyrolysis chamber to be isolated for inserting and removing samples and to introduce a purge flow to remove air prior to heating. Applications of pyrolysis include art materials, biological samples, environmental samples, food and agriculture, forensic analysis, geochemistry, and fuels and synthetic polymers. An example illustrating the pyrolysis of isoprene-butadiene rubber is shown in Figure 11.36. Wampler recently provided a thorough review of pyrolysis GC, written in an excellent, introductory style (124).

11.6.6 Automation

Automation is an essential part of the sample preparation procedures. The added imprecision that results from the increased manipulation of the sample during extraction can be minimized by the use of robotics and automated sample preparation systems. For these reasons, it is essential that the validation of analytical methods include sample preparation. The validation process defines the method parameters and the impact that each parameter has on the effectiveness of the extraction method. In addition, the use of manual techniques is not practical

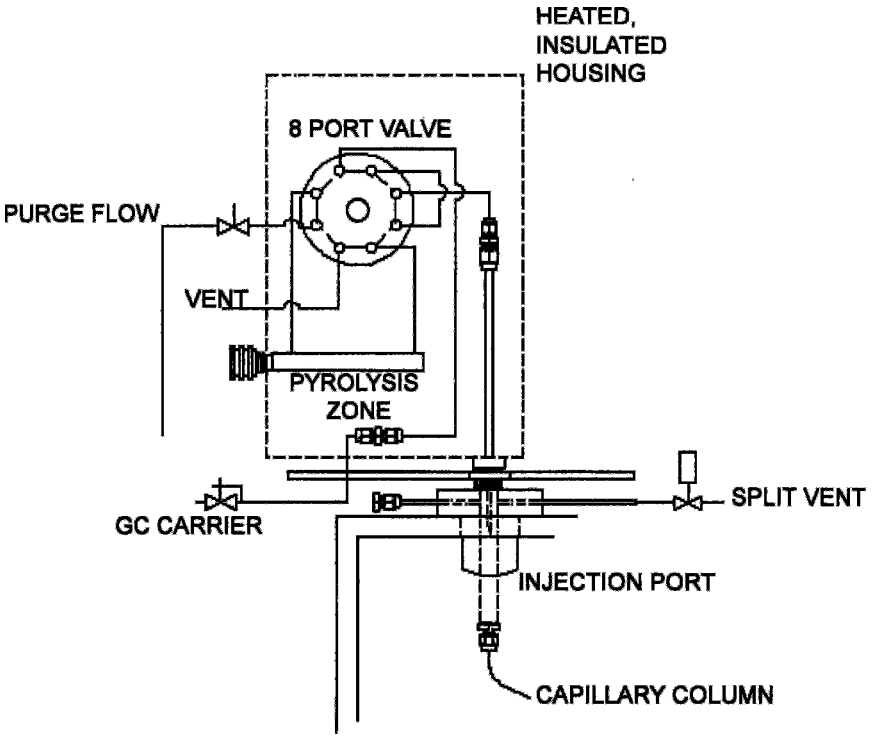

FIGURE 11.35 Instrumentation for pyrolysis gas chromatography [reprinted with permission from T. P. Wampler, *J. Chromatogr. A* **842** 207–220 (1999), Figure 3].

for today's high-throughput research and quality control labs. The importance of automated equipment is evident by the nearly extinct practice of manual injection in industrial laboratories.

11.6.7 Derivatization

For the gas chromatographic analysis of polar or nonvolatile analytes, derivatization is often necessary. Usually, derivatization is performed to replace a polar functional group that may cause the native analyte to be either reactive or nonvolatile, with a nonpolar group, making the resulting product less reactive or more volatile. Also, derivatization with an optically active derivatizing reagent may be performed to resolve chiral molecules into diastereomers, which can be separated on traditional columns. For reducing polarity, the common methods include alkylation by esterification using silylating or acylating reagents. Alkyl esters are generally very stable and perform well chromatographically. For chiral derivatization, the derivatizing reagents are designed to target specific functional groups and must be enantiomerically pure. Derivatization is often used in drugs of abuse, pharmaceutical, environmental, and food analysis and there is

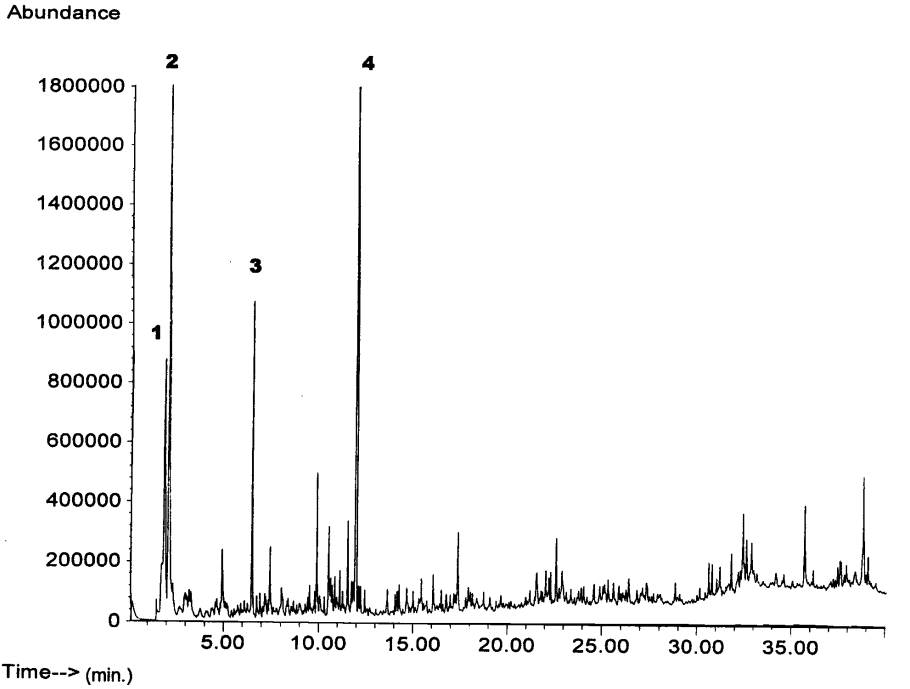

FIGURE 11.36 Gas chromatogram showing pyrolysis of isoprene–butadiene rubber [1 = butadiene, 2 = isoprene, 3 = butadiene dimmer, 4 = limonene (isoprene dimmer)]. Conditions: pyrolyzer—CDS Analytical Model 2500, 750°C, 20 s; interface oven 300°C, GC—6890 (Agilent), column HP-5, 30 m × 0.25 mm. Carrier gas: helium, 5.9 psi, split ratio 75–1, temperature program 40°C, 2 min, 6°C/min to 295°C. Detector: mass selective detector. [Reprinted with permission from T. P. Wampler, *J. Chromatogr. A* **842**, 207–220 (1999), Figure 7.].

a tremendous body of literature. The text by Knapp (125) is considered the classical reference. Several of the vendors of derivatization reagents have extensive reference materials, both printed (126) on the World Wide Web (127).

11.6.8 Thermal Desorption

Thermal desorption and related techniques have been described as akin to using temperature as a syringe (128). In this chapter, several techniques that may be described as thermal desorption have been described, including headspace sampling, solid-phase microextraction, and the other sorbent-based extraction methods, plus SFE with sorbent trapping, as the sorbent must be desorbed into the chromatograph. In all thermal desorption techniques, sample is swept into the gas chromatograph using the heating and the flow of carrier gas. In all cases, the introduction of the sample as a narrow chromatographic band is critical.

If the sample is small enough or the sorbent on which it is trapped is of sufficiently low thermal mass, the sample can be heated rapidly. Commonly, however, sample heating is slow enough that the desorbed material must be cold-trapped at the head of the column by the use of either a cold trap or a cryogenic oven. This is essentially a two-step desorption: (1) the sample is desorbed from the sorbent trap and (2) it is recondensed on the column and is desorbed as the temperature program proceeds. There are numerous configurations and types of equipment available for thermal desorption. These have been recently reviewed by Hinshaw (129) and Wampler (130).

11.7 CONCLUSIONS

Sample preparation for gas chromatography is an extremely broad and sometimes complex field. This owes itself to the tremendous versatility of GC, with applications and sample types nearly as numerous as analysts themselves. The choice and optimization of sample preparation is often the most difficult part of method development for gas chromatography. Often the choice is made by the instrumentation that is already available in the laboratory, and in many cases this is not always the best technique. Instrumental and automated sample preparation techniques saw tremendous growth in the 1990s, although the advent of these modern systems has not lessened the need for analysts to approach sample preparation for sound science. Nearly all sample preparation techniques involve the transfer of analyte from one phase to another. Despite the growth in automated techniques, the fundamental phase equilibrium principles presented in this chapter still form the basis for all techniques involving phase transfer. In this chapter, we have attempted to provide an overview of the major sample preparation techniques for gas chromatography. We have provided an overview of each technique, along with basic theory and applications. We have attempted to heavily reference each technique, including reference to the most recent review articles in the research literature. The reader is strongly encouraged to obtain and peruse these references to obtain more details. Sample preparation has always been and remains today a very active area of research by gas chromatographers.

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CHAPTER TWELVE

Physicochemical Measurements by Gas Chromatography

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Gas chromatography (GC) is generally considered as a means of analysis of complex mixtures. The subtle differences among the interactions of the components of a mixture with the stationary phase in the gas chromatographic column are the primary features that make GC such a useful technique. The goal of a gas chromatographic experiment is often, therefore, stated as the separation, identification, and quantification of the components, with only passing regard to the nature of this process. However, the fundamental physical and chemical properties of the chromatographic system and the materials that constitute it are amenable to investigation by gas chromatographic experiments. The sensitivity to subtle differences in molecular structure and detail that has been exploited in its use in separation is also exhibited in the detailed information that GC can give on the physicochemical properties of the stationary phase and the materials injected onto the column. Although the measurements of physical parameters such as specific surface area and heat of adsorption are often available from other experiments, gas chromatographic techniques have advantages of speed, reliability, and versatility, in that one apparatus may be configured to measure physicochemical properties over a wide range of conditions. Advances in gas chromatographic hardware, especially columns and detection systems, have made rapid measurements possible on very small quantities of sample, making GC an even more powerful tool for physicochemical measurements.

12.1 GAS–SOLID CHROMATOGRAPHY, ADSORPTION, AND SPECIFIC SURFACE AREA

Gas–liquid chromatography has been the focus of much discussion of GC. It is often the case that development of hardware for GC focuses on removing interactions with solid surfaces because the surfaces are considered to be sources of undesirable adsorption sites that contribute to peak asymmetry, especially those in the injection port liner or the chromatographic column. However, interactions between a solid substrate and a gas (mobile) phase are also informative. When a solid without a liquid substrate is solely and deliberately used inside the chromatographic column, the technique is called *gas–solid chromatography* (GSC).

The definitions in gas–solid chromatography are modifications of the definitions and terms used in gas–liquid chromatography. The phase ratio is:

$$\beta = \frac{V_G}{V_A} \tag{12.1}$$

where β is the phase ratio, V_G is the interstitial mobile-phase volume, and V_A is the true adsorbent volume (weight of adsorbent/density $= V_A$).

In the rate theory of gas–solid chromatography, the equation for H, the height equivalent to a theoretical plate, is essentially the same as for gas–liquid chromatography except that C_k replaces C_l (resistance to mass transfer in the liquid

phase); C_k is a term characteristic of adsorption kinetics.

$$H = \sum_{i=1}^{5} \frac{1}{\frac{1}{A} + \frac{1}{C_1 u}} + \frac{B}{u} + C_k u + C_g + H_l \tag{12.2}$$

Theoretical considerations indicate that, on a homogeneous surface, C_k is smaller than C_1.

One considerable obstacle to the use of gas–solid chromatography is a general lack of adequate descriptions of adsorbent structure, including information on the distribution and dimensions of pores. In addition, the lack of reproducibility of adsorbents, not only among manufacturers (different products, presumably the same) but also within the same manufacturer (different lots), provides a variability that hinders reproducible separation properties.

12.1.1 Adsorbent Properties

A solid adsorbent's properties determine the effects one observes in gas–solid chromatography. The specific surface area (m^2/g) is important. The greater the surface area, the higher the probability of some sorption process occurring. Also, the more active sites per unit area, the more reactive are the sorbate molecules with the sorbent surface. The chemical composition of the surface layer and its crystal structure are of interest. Knowledge of composition permits one to articulate the types of wanted or unwanted reactions that may take place at the gas–solid interface. Finally, knowledge of the pore structure is helpful in identifying molecules that may selectively be trapped or sorbed on the surface. Primary information regarding adsorption phenomena comes from the analysis of adsorption isotherms.

12.1.2 Adsorption of Gases at Solid Surface

Regardless of the process at the gas–solid surface, physical adsorption of the gas or vapor on the solid surface is part of the mechanism. The distance between sorbed molecules is shorter than the distances found between molecules of a real gas, but these distances are larger than those encountered in chemical interactions. The interaction energy between molecules and the surface of a sorbent may be estimated by assuming that the molecules are spherical in shape and located in a field of infinite sorbent. It is usual to consider the temperature to be sufficiently high to reduce the importance of molecular interactions to a negligible value. By introducing N gas molecules at a temperature T and a pressure P into a container holding a sorbent with a uniform surface, one can calculate the "apparent volume" V_a from the ideal-gas law:

$$V_a = \frac{NkT}{P} \tag{12.3}$$

This neglects the effects of adsorption. If V_0 is determined from the limiting value of $1/V_a$ as P goes to zero,

$$\lim_{P \to 0} \frac{1}{V_a} = \frac{1}{V_0} = \frac{P V_a C}{kT V_0^3} \tag{12.4}$$

one may express this limiting volume by accounting for those molecules that are adsorbed:

$$V_0 = V_m + \frac{1}{V} \int V_m \exp \frac{-E}{kT} \, dV \tag{12.5}$$

V_m represents the "void" volume of the uniform sorbent surface, E is the potential energy of gas molecules in the sorbent field, and $1/V_0$ is the intercept of the linear plot of $1/V_0$ versus PV_a. The value of E may then be calculated from integration of Equation 12.5 over all volumes.

The carrier gas may have a significant effect on the separation process. Adsorbents with high specific surface areas adsorb the carrier gas, thus decreasing the number of the active sites (adsorption centers) available to the component of interest. This results in a change of component adsorption, which is in proportion to the adsorption capacity of the carrier gas. A change in carrier gas from hydrogen or helium to nitrogen may produce sharper peaks because of the higher adsorption capacity for nitrogen (1).

Sorbates (solute molecules) may be grouped according to their intermolecular interactions. These groupings are based on electronic configurations, electron density, and functional groups in the molecule:

1. *Group A Molecules*. These molecules have spherically symmetric electron shells. Examples are noble gases and the saturated hydrocarbons, which have only sigma bonds between the carbon atoms. Molecules of this type interact nonspecifically, through dispersive forces resulting from concordant electronic motion in the interacting molecules.
2. *Group B Molecules*. These molecules have a concentrated electron density (negative charge)(e.g., unsaturated aromatic hydrocarbons) and/or π-electron bonds (e.g., N_2, H_2O, ROH, RCOR, NH_3, NHR2, NR_3, RSH, RCN).
3. *Group C Molecules*. These include molecules with locally concentrated positive charges within small-radius linkages, but these should not be adjacent groups with concentrated electron densities (e.g., –OH or =NH groups). Organometallic compounds exemplify this group. This type of compound interacts specifically with group B molecules but nonspecifically with group A molecules.
4. *Group D Molecules*. These molecules have adjacent bonds, one with a positive charge and one with electron density. Molecules with –OH and =NH functional groups, for example, H_2O, ROH, and 1° and 2° amines, constitute this group. Group D molecules may interact specifically with

group B and C molecules and with each other; however, they interact nonspecifically with group A molecules.

Adsorbents are classified as either specific or nonspecific. The specificity results from the type of molecule or functional group attached to the adsorbent surface. Three classifications result:

1. *Nonspecific Adsorbents*. There are no functional groups or exchangeable ions on the surface of these adsorbents. Adsorbents of this type are carbon black, boron nitride, and polymeric saturated hydrocarbons (e.g., polyethylene).
2. *Specific Adsorbents with Positive Surface Charges*. These adsorbents have acidic hydroxyl groups (hydroxylated acid oxides such as silica), aprotic acid centers, or small-radius cations (zeolites) on the surface. Adsorbents of this type interact with molecules that have locally concentrated electron densities, that is, group B and group D molecules.
3. *Specific Adsorbents with Electron Densities on the Surface*. Graphitized carbon blacks with dense monolayers or group B molecules or macromolecules on the surface are found in this group. Adsorbents with a functional group, for example, cyano, nitrile, or carbonyl, are also included in this category.

Adsorbents may also be classified according to their structure. These classifications are summarized in Table 12.1.

In gas–solid chromatography, retention of sorbate compounds is determined by (1) the chemical nature and geometric pore structure of the sorbent, (2) the molecular weight of the sorbate molecules and their geometric and electronic structures, and (3) the temperature of the column.

Column separating power depends on selectivity of the sorbent and diffuseness or spreading of chromatographic bands moving through the sorbent. Thus

TABLE 12.1 Adsorbents, Classified by Type

Type	Description and Classification
I—Nonporous	Nonporous mono- and polycrystalline sorbents (e.g., graphitized carbon black, NaCl); porous amorphous sorbents (e.g., Aerosil and thermal blacks)
II—Uniform wide pores	Large-pore glasses, wide-pore Xerogels, and compressed powders made from nonporous particles (>100 Å in size and specific surface areas <300 m^2/g)
III—Uniform fine pores	Amorphous fine-pore xerogels, fine-pore glasses, many activated charcoals, and porous crystals (type A and X zeolites)
IV—Nonuniform pores	Chalklike silicagels obtained from hydrolysis of salts from strong acids in a silicate solution

any chromatographic column is most effective when bands are diffuse. Band spreading is caused by thermodynamic, kinetic, injection, and diffusional effects. These may be summarized as follows:

1. Nonsymmetric band spreading may be attributed to nonlinearity of the equilibrium adsorption isotherm (i.e., deviation of the isotherm from Henry's law). This causes the sorbate to move through the column at different rates (rate dependent on sorbate concentration).
2. Peak diffuseness can be attributed to various processes occurring during the transport of the sorbate through the column. Diffusion processes are very complex due to (a) ordinary diffusion in the gas phase; (b) band movement through particle layers of different sizes and shapes and packed in various ways, causing diffuseness related to a nonuniform distribution of gas flowrates over each cross-sectional area; and (c) difference in local flow velocities from the average velocity through the column. Columns may exhibit what is referred to as the *wall effect*, which implies that the flow at the walls is higher than the average of the column because resistance at the wall is less. The great effectiveness of capillary columns is due mainly to the absence of specific diffusional processes caused by particles. However, one does observe diffuseness in capillary or unpacked columns from the parabolic velocity distribution over the column cross section. The gas velocity is higher at the center and lower near the walls than the average velocity of the band.
3. Peak diffuseness may be a result of the kinetics of the sorption-desorption process (i.e., slow mass transfer or exchange at sorbent surfaces). Peak diffusion in this case is usually nonsymmetric because the rates of sorption and desorption are not the same. Band spreading due to the final rate of mass exchange is closely related to the diffusion phenomenon. Physical adsorption, for all practical purposes, is instantaneous. The overall process of sorption, however, consists of several parts: (a) the movement of sorbate molecules toward the sorbent surface, resulting from intergrain diffusion (outer diffusion), (b) movement of sorbate molecules to the inside of pores (i.e., internal diffusion of the sorbate molecules in the pores and surface diffusion in the pores), and (c) the sorption process in general.
4. Apparent diffusion may be influenced by the time it takes for sample injection.

Some common adsorbents used for gas–solid chromatography are given below. [Some of these materials are commercially available as porous-layer open tubular columns (PLOT). PLOT columns yield higher sensitivity than do the traditional packed columns because of their higher column permeability and lower pressure drop (than packed columns).]

1. *Carbon*. These are nonspecific-type adsorbents because of the lack of functional groups, ions, or unsaturated bonds. Most interactions are due to dispersive forces.

2. *Metal Oxides*. These include (a) silicagel, which is a specific type of adsorbent because there are free hydroxyl groups on the surface (polar molecules are easily separated on these materials, and wide-pore silicas with homogeneous surfaces are used for analytical gas–solid chromatography;) and (b) alumina. These columns can be easily baked out to remove contaminants and reused with good reproducibility.
3. *Zeolites*. Zeolites are useful in gas–solid chromatography because of their pore structure and good adsorption properties. They are a specific type of adsorbent, with cavities allowing sieving action for low-molecular-weight molecules to enter "holes" (windows).
4. *Inorganic Adsorbents*. These have two general classifications: (a) inorganic salts [e.g., alkali metal nitrates and halides (2), alkaline earth halides (3), vanadium, manganese, and cobalt chlorides (4), and barium salts (5)] and (b) inorganic salts, coated on surfaces of silica, alumina, carbon, and so on.
5. *Organic Adsorbents*. These include (a) organic crystalline compounds, (b) liquid phases below their melting point, (c) organic clay derivatives (e.g., Bentone 34), and porous polymers.

12.1.3 Specific Surface Area

The specific surface area of a solid is the quantity of surface available for a particular application, per unit weight, usually given as m^2/g or, if volumetric, m^2/mL. The specific surface area is one of the quantities that must be known if any physicochemical interpretation of the behavior of the material as an adsorbent is to be made. Typical specific surface areas of some gas chromatographic packings and supports are given in Table 12.2 (6).

The best-known method for determining the specific surface area of powders was developed by Brunnauer, Emmett, and Teller (BET) (7). The BET method involves the determination of the quantity of gas taken up through adsorption by a solid adsorbent at equilibrium with a gas phase at a pressure P. After a known quantity of gas has been admitted to a chamber containing the sample, adsorption occurs, resulting in a pressure decrease until equilibrium between the adsorbed and gas phases is reached. The quantity of gas adsorbed is determined by the difference between the amount of gas originally admitted and the amount remaining in the gas phase at equilibrium. The quantity of gas originally admitted is calculable from the initial pressure because the volume above the adsorbent was calculated previously.

The amount of gas adsorbed depends on the pressure of the gas with which it is in equilibrium. Thus determination of the equilibrium for various amounts of gas originally admitted gives different equilibrium pressures and amount adsorbed.

TABLE 12.2 Specific Surface Areas of Some Common Gas Chromatographic Supports and Packings

Packing	Specific Surface Area (m^2/g)	Packing	Specific Surface Area (m^2/g)
Alumina F-1	223	Chromosorb 750	0.8
Carbopack A	13.2	Molecular sieve A	230
Carbopack B	81.9	Molecular sieve 13X	91.2
Carbosieve B	615	Porapak N	518
Chromosorb T	6.94	Porapak P	119
Chromosorb W	1.0	Porapak Q	515
Chromosorb 101	35.4	Porapak R	544
Chromosorb 102	33.8	Porapak S	411
Chromosorb 103	20.3	Porapak T	266
Chromosorb 104	122	Porapak PS	108
Chromosorb 105	452	Porapak QS	453
Chromosorb 106	764	Silicagel	673
Chromosorb 107	416		
Chromosorb 108	162		

Source: Reference 6.

These two parameters are related by the BET equation:

$$\frac{P}{V_a(P_0 - P)} = \frac{(C-1)}{V_m C P_0} P + \frac{1}{V_m C} \tag{12.6}$$

where V_a is the volume of gas adsorbed [reduced to standard temperature and pressure (STP)] per gram of adsorbent, P is the equilibrium pressure, P_0 is the saturation vapor pressure of the adsorbate at the temperature of adsorption, C is a constant determined by the energy of adsorption, and V_m is the volume (at STP) of gas (per gram of adsorbent) that fills one monolayer.

A plot of $P/[V_a(P_0 - P)]$ versus P/P_0 allows one to determine V_m from the slope and intercept. Once known, this value may be converted to a specific surface area S, in m^2/g, if one knows the average area occupied by one molecule by the following equation:

$$S = \frac{V_m \sigma N}{V_0} \tag{12.7}$$

where σ is the average area occupied by one molecule (in m^2/molecule), N is Avogadro's number (6.022×10^{23} molecules/mol), and V_0 is the STP volume of one mole of gas (22.410 L/mol).

12.1.4 Gas Chromatographic Surface Area Determination

Gas chromatographic determination of specific surface area has several advantages over the traditional BET adsorption method. It is more accurate when the

adsorption involves low surface coverage and can be readily adapted to reflect conditions other than those near the condensation point; for example, contact time and temperature may be easily varied. The gas chromatographic measurement is usually more convenient to set up and use.

The first demonstration of the use of GC to determine specific surface area was made by Nelsen and Eggertsen (N/E) (8). In their method, a quantity of the adsorbent is outgassed at high temperature under helium flow to remove volatile components. (This procedure may do more than remove volatile constituents; if the conditions are sufficiently severe, outgassing may actually alter the structure of the surface.) After cooling to room temperature, the sample, usually in a U tube, is connected to the chromatograph. A flow of helium and nitrogen is sent over the sample. After the gas composition is stable, indicated by no change of the gas chromatographic detector response, the sample is immersed in a liquid nitrogen bath. Adsorption of nitrogen from the gas stream occurs, causing the thermal conductivity detector to show what is known as an *adsorption peak* (Figure 12.1). The sample comes to equilibrium at this temperature, indicated by a settling of the detector response. Removal of the liquid nitrogen bath results in desorption of the previously adsorbed material. The gas chromatographic detector indicates this with a *desorption peak.* The desorption peak's area determines the amount desorbed, if the detector had been previously calibrated with known amounts of nitrogen. Although the adsorption peak could, in principle, be used to measure the same effect, tailing of the adsorption peak makes calculation of the amount adsorbed from the desorption peak preferable. This adsorption corresponds to a nitrogen pressure with which the adsorbed phase is in equilibrium. This partial pressure is calculable from the total flowrate F_c, the nitrogen flowrate F_N, and the barometric pressure P_B:

$$P = \frac{F_N}{F_c} P_B \tag{12.8}$$

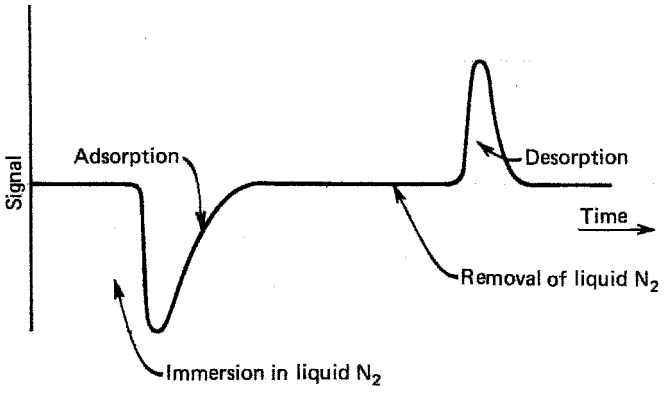

FIGURE 12.1 Schematic detector response in the N/E determination of specific surface area.

The measurement is repeated for different values of the nitrogen flowrate to establish the isotherm, which may be plotted and analyzed by applying Equations (12.6) and (12.7) to obtain the specific surface area.

The N/E method is used for a variety of adsorbents. Improvements to the method extend the range to the measurement of low specific surface areas (down to 0.07 m^2/g), such as by stop-flow techniques. Table 12.3 gives comparisons of specific surface areas determined by the N/E method and by the classical BET method, which indicate that the agreement between the two is quite good.

The shape of the front may be related to the adsorption process. For example, Kuge and Yoshikawa (9) relate peak shape to the beginning of multilayer adsorption (Figure 12.2). At injections of very low volume, the peak is symmetric; however, injections of larger volumes produces a peak with a sharp front, a diffuse tail, and a defect at the front of the top of the peak. For extremely large injections, the peak has a rather diffuse front and a sharp tail. By using repeated injections, those authors were able to determine the injection volume for which the transition from one behavior to another occurs. This corresponds to point *B* on a BET type II isotherm, from which the authors were able to calculate the specific surface area. A complete analysis of the front can enable one to determine the adsorption isotherms, and hence specific surface area (10). The description by Kiselev and Yashin (11) is particularly elegant, and we briefly recapitulate it here.

Under certain conditions, the dominant source of broadening of peaks in a gas chromatographic experiment is the adsorption–desorption process. Consider a small volume of gas moving through a packed column. In this volume is a certain concentration of the substance to be sampled by the detector. As it moves through the column, the concentration is changed in one of two ways: (1) by net flow of the substance into or out of this small volume from the nearby gas phase or (2) by the adsorption–desorption processes, which transfer material from the adsorbed phase to the gas phase. The net result of these two processes is that, at equilibrium, the changes in the gas-phase concentration c and the adsorbed-phase

TABLE 12.3 Comparison of Surface Area Using BET and N/E Methods

Sample	BET (m^2/g)	N/E (m^2/g)
Furnace Black	24	25.7
Silica–alumina cracking catalyst, used	103	101
Silica–alumina cracking catalyst, new	438	455
Alumina	237	231
Firebrick	3.1	3.4

Source: Reprinted from Reference 3 with permission. Copyright by the American Chemical Society.

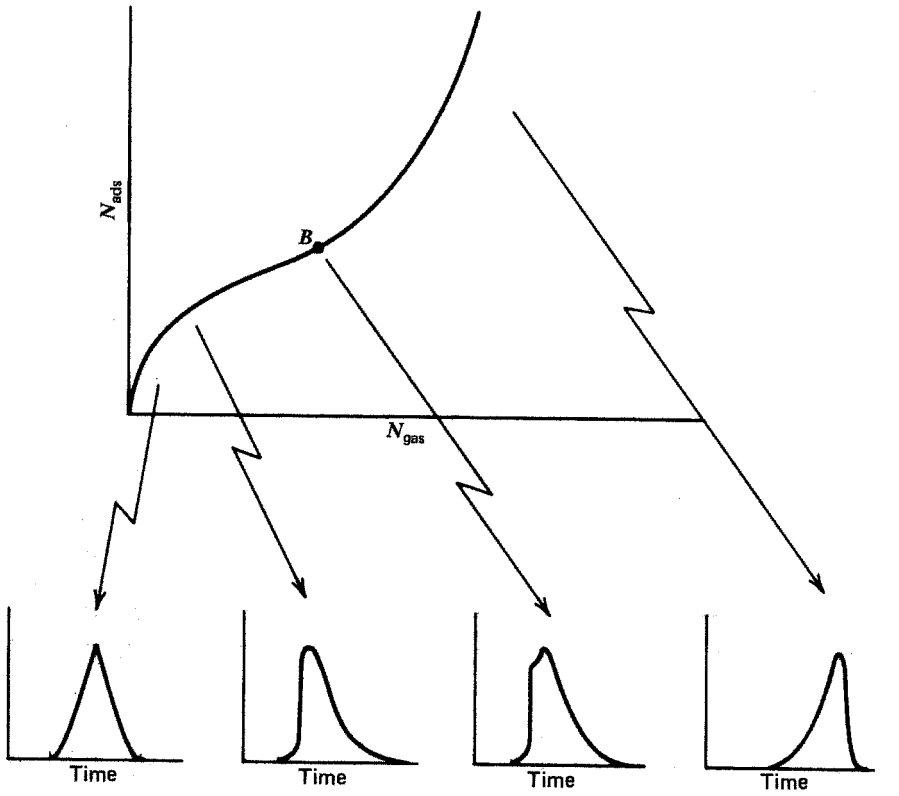

FIGURE 12.2 Peak shape in a multilayer adsorption isotherm. (From Reference 4).

concentration c_a are related by

$$\left[\frac{\delta c_a}{\delta c}\right]_x = \left[\frac{F_c - F}{F}\right]\frac{V}{V_a} \tag{12.9}$$

where F_c is the flowrate of carrier gas, F is the flowrate of a volume of concentration c, V is the gas-phase volume, and V_a is the adsorbed-phase volume. Uptake is usually expressed not as a volumetric concentration, but as a quantity taken up per gram of adsorbent a. The result of conversion is Equation 12.10:

$$\left[\frac{\delta a}{\delta c}\right]_x = \frac{V_R'}{m} \tag{12.10}$$

where

$$a(c) = \frac{1}{m}\int_0^c V_R'(c')dc' \tag{12.11}$$

where V_R' is the adjusted retention volume and m is the mass of the adsorbent. To find the uptake a from a gas phase of concentration c, therefore, one integrates Equation 12.10. Equation 12.11 is the relationship needed to express uptake in terms of the gas chromatographic observables. If the detector is concentration-sensitive, the integral of Equation 12.11 can be evaluated directly from the chromatogram, since the height y of the chromatographic detector response corresponds to a given gas-phase concentration. A few algebraic manipulations lead to Equation 12.12:

$$a(c) = \frac{M_a}{m_a m A} \int_0^y \{t_c(y') - t_0\}\, dy' \tag{12.12}$$

where M_a is the molecular weight of the adsorbate, m_a is the mass of adsorbate injected, and A is defined as follows:

$$A = \int_0^\infty y(t')\, dt' \tag{12.13}$$

These integrals are shown in Figure 12.3. The pressure to which this uptake corresponds is calculable, assuming an ideal gas phase, as

$$P = \frac{m_a y R T}{M_a F_c A} \tag{12.14}$$

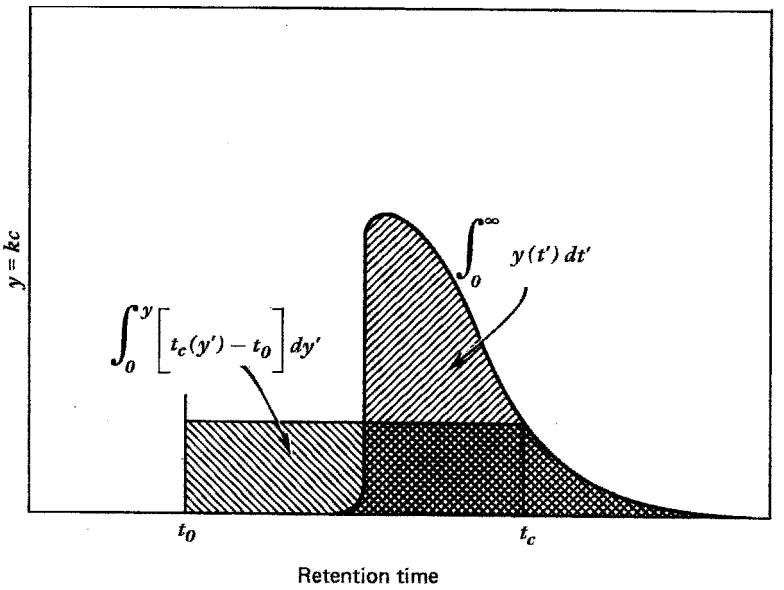

FIGURE 12.3 Schematic of a chromatographic response, showing the areas corresponding to the integrals in Equation 12.7.

where R is the gas constant and T is the absolute temperature. One may, therefore, construct the adsorption isotherm from such an analysis.

Both permanent gases and adsorbates that are liquids above room temperature can be investigated by this method. With the use of specific detectors, gas chromatographic analysis can provide unique adsorption data on interesting systems, including the study of adsorption of hydrocarbons with the use of a flame ionization detector (FID).

Various types of surface area may be determined by GC. For example, the adjusted retention time of a probe molecule, such as *n*-octane, is fairly linear in the weight of polymer adsorbent in a coated column, so a plot of retention time versus weight in such a column can give the specific surface area from the slope of the plot. In catalyst surface area determinations, the emphasis is usually on measurement of catalytically active surface area rather than on the total surface area. Commonly, chemisorption of gases such as hydrogen, oxygen, or carbon monoxide is used to measure the "active surface area" in a Langmuir adsorption experiment. Karnaukov and Buyanova (12) developed a gas chromatographic method for determining the active surface area of a complex catalyst, by repeated small injections until breakthrough was observed. The total volume chemisorbed is the total volume injected up to breakthrough. Masukawa and Kobayashi (13) used a packed column to determine the "size" of an adsorbate in the adsorbed state. The method is time-consuming, but estimates of the "size" of the molecule according to a theoretical model provide information on the structure of the adsorbent. Hamieh and Schultz (14) developed a method to determine the surface areas of polar molecules using a combination of dynamic contact angle and inverse gas chromatographic techniques. ZnO, MgO, and polytetrafluoroethylene were studied and the results compared to some theoretical models.

There are many sources of error in surface area determinations. Lobenstein (15) has delineated the problems associated with lack of additivity, as applied to coadsorption of mixtures, and has derived equations for interactions among coadsorbates to give a more realistic value for surface areas under the conditions of coadsorption. Dollimore and Heal (16) have shown that it is possible to calculate an apparent specific surface area that is less than the actual area because the adsorbate fits snugly in pores of the adsorbent. Thus changes in the "size" of the adsorbate molecule change the surface area determined, with the "true" surface tending to be that found for larger adsorbates. In using flow techniques, care must be taken to ensure that repeated injections are performed with all parameters controlled. The flowrate is an exceptionally critical parameter. Since specific surface area depends on the probes used to measure it, the analyst should always measure the area under conditions that mimic as closely as possible the conditions under which the adsorbent will be used.

12.2 SURFACE THERMODYNAMICS

Adsorption isotherms constructed from gas chromatographic experiments of the type discussed in Section 12.1 contain substantially more information on

the adsorption–desorption behavior than the specific surface area. The use of temperature as a parameter yields information on the thermodynamics of the adsorption–desorption process. A growing body of data on adsorption and thermodynamics (17) provides the basis for predicting sample separations under a variety of conditions, a goal of separation science.

One of the most important of the thermodynamic parameters measurable by gas chromatographic techniques is the isosteric heat of adsorption ΔH_{st}°. Measurements of heats of adsorption provide a quantification of the interactions occurring between adsorbate and adsorbent. Although there exists a spectrum of behaviors, the process of adsorption can be roughly divided into three categories: physical adsorption due to weak van der Waals interactions, reversible chemical adsorption, and irreversible chemical adsorption. The last two types occur because of a much stronger specific interaction, such as that of unsaturated hydrocarbons with transition metals or the adsorption of water on activated alumina. It is usual to distinguish physical and chemical adsorption on the basis of the enthalpy of adsorption with a typical value of 15 kcal/mol (62.8 kJ/mol) serving as the arbitrary dividing line between physical and chemical adsorption. Measurements of heats of adsorption from a flow system, as is done in GC, result from the equilibrium that exists between the gas phase and the adsorbed phase.

The process of adsorption–desorption occurring in the gas chromatograph is written as

$$A(\text{gas}; P, T) \leftrightarrow A(\text{adsorbed}; a, T)$$

The equilibrium constant K_D for this process is given by Equation 12.15:

$$\ln K_D = -\frac{\overline{\Delta G^{\circ}}}{RT} \tag{12.15}$$

From the relationship between the free energy and enthalpy of adsorption, one obtains a very useful result:

$$\left[\frac{\partial \ln P}{\partial (1/T)}\right]_a = \frac{\overline{\Delta H_{st}^{\circ}}}{R} \tag{12.16}$$

A plot of ln P versus $1/T$ at constant coverage a gives a straight line, the slope of which is the isosteric enthalpy of adsorption divided by the gas constant. This enthalpy change is *isosteric*, in that it is evaluated at constant coverage. The prescription for a determination is as follows:

1. Determine the isotherms as in Section 12.1 for several temperatures.
2. For a given isostere (cross section at constant coverage), plot ln P versus $1/T$.
3. Determine ΔH_{st}° from the slope of this plot.

The enthalpy change determined in this way depends on the value of a, the coverage; hence the coverage dependence of ΔH_{st}° can be examined from the

variation of the slope with the value of a. This is a useful indicator of the state of the adsorbed system since surface heterogeneity and the magnitude of the direct interaction of adsorbates produce this coverage dependence. In principle, gas chromatographic determination of this dependence on coverage gives detailed information on the subtle features of surface interactions.

Further thermodynamic parameters may be calculated from the temperature and coverage dependence of $\Delta H^\circ(a, T)$. From thermodynamics, one obtains Equation 12.17, which relates $\Delta G^\circ(a, T)$ to $\Delta H^\circ(a, T)$ (18):

$$\left[\frac{\partial \overline{\Delta G^\circ}/T}{\partial T}\right]_{P,a} = -\frac{\overline{\Delta H^\circ}(a, T)}{T^2} \tag{12.17}$$

The standard free energy for adsorption is then at constant coverage):

$$\overline{\Delta G^\circ}(a, T) = \overline{\Delta G^\circ}(a, T_0) - T\int_{T_0}^{T} \frac{\overline{\Delta H^\circ}(a, T')}{T'^2}\, dT' \tag{12.18}$$

Once $\Delta H^\circ(a, T)$ and $\Delta G^\circ(a, T)$ are known, the entropy of adsorption can be directly calculated by the thermodynamic relationship:

$$\overline{\Delta S^\circ}(a, T) = \frac{\overline{\Delta H^\circ}(a, T) - \overline{\Delta G^\circ}(a, T)}{T} \tag{12.19}$$

where T_0 is a temperature for which the standard free energy of adsorption is defined. The use of GC to yield these thermodynamic parameters has been exploited (16) and should lead to an understanding of the processes underlying the separation process.

Frequently, the stationary phases used in gas chromatographic separations are much more uniform than the general theory given in Section 12.1 implies. Additionally, the amount of material injected is extremely small, corresponding to very low surface coverage. The resultant chromatogram shows little band broadening from adsorption–desorption effects and the retention of the injected material corresponds to the case of an infinitely dilute adsorbate in the stationary phase. The distribution constant K_D may be related to the retention volume, the mobile-phase volume, and the stationary-phase volume. The total number of moles injected onto the column n is divided between the stationary and mobile phases

$$n = n_s + n_M \tag{12.20}$$

where n_s $(= ma)$ is the total number of moles in the stationary phase and n_M is the number of moles in the mobile phase. The distribution constant K_D is defined as

$$K_D = \frac{a_s}{a_M} = \frac{\gamma_s}{\gamma_M}\frac{n_s/V_s}{n_M/V_M} \tag{12.21}$$

where a_s is the activity in the stationary phase, a_M is the activity in the mobile phase, V_s is the volume of the stationary phase, V_M is the volume of the mobile phase between the point of injection and the point of detection, γ_s is the activity coefficient in the stationary phase, and γ_M is the activity coefficient of the adsorbate in the mobile phase. The velocity of the substance u_s is related to the velocity of an unretained substance u_u by the following equation:

$$u_s = u_u f \tag{12.22}$$

where f represents the fraction of time spent by the substance in the mobile phase. On average, f is equivalent to n_M/n and

$$u_s = \frac{n_M}{n} u_u \tag{12.23}$$

By substitution

$$\frac{n_M}{n} = \frac{n_M}{n_s + n_M} \tag{12.24}$$

$$\frac{n_s}{n} = n_M K_D \left(\frac{V_s}{V_M}\right)\left(\frac{\gamma_M}{\gamma_s}\right) \tag{12.25}$$

$$\frac{n_M}{n} = \frac{1}{1 + (K_D V_s \gamma_M / V_M \gamma_s)} \tag{12.26}$$

The average velocity of an unretained component u_u and the average velocity of the substance u_s are related to the flowrate of the carrier gas F_c and the column length L by the following expressions:

$$u_u = \frac{F_c L}{V_M} \tag{12.27}$$

$$u_s = \frac{F_c L}{V_M + K_D V_s (\gamma_M/\gamma_s)} \tag{12.28}$$

The average velocity of the substance is also defined by the following expression:

$$u_s = \frac{F_c L}{V_R} \tag{12.29}$$

where V_R is the retention volume of the substance. Therefore, from Equation 12.21, we obtain

$$K_D + \frac{(V_R - V_M)\gamma_s}{V_s \gamma_M} = \frac{V_R'}{V_s}\frac{\gamma_s}{\gamma_M} \tag{12.30}$$

where V_R' is the adjusted retention volume. This equation is similar in form to Equation 12.12, assuming that all molecules have exactly the same retention volume.

The volume of the stationary phase remains constant; thus variations of K_D with temperature are reflective of variations of V_R', γ_s, and γ_M with temperature. Equation 12.15 gives such a relationship:

$$\ln V_R' - \ln V_s = -\frac{\overline{\Delta G^\circ}}{RT} - \ln \frac{\gamma_s}{\gamma_M} \tag{12.31}$$

Differentiation of Equation 12.31 with respect to $1/T$ and comparison with Equation 12.17 gives the important result:

$$\left[\frac{\partial \ln V_{R'}}{\partial(1/T)}\right]_P = \frac{\overline{\Delta H_{st}^\circ}}{R}(a = 0) \tag{12.32}$$

The enthalpy of adsorption corresponds to infinite dilution on a homogeneous stationary phase. Thus, for sufficiently homogeneous stationary phases (whether they are solid or nonvolatile liquid is not germane to the treatment), the slope of a plot of the adjusted retention volume (V_R') versus $1/T$ can be used to calculate ΔH° at infinite dilution. Because the value of $\ln V_s$ is found from the intercept of such a plot, ΔG° can be calculated by using Equation 12.31 and ΔS° is then calculable from Equation 12.19.

To account for hydrodynamic factors due to a finite pressure drop over the length of the column, the adjusted retention volume is corrected by the pressure gradient correction factor j to give the net retention volume V_N

$$V_N = jV_R' \tag{12.33}$$

and

$$j = \left[\frac{3}{2}\frac{(P_i/P_o)^2 - 1}{(P_i/P_o)^3 - 1}\right] \tag{12.34}$$

where P_i is the pressure of the inlet and P_o is the pressure at the outlet. As long as the pressures are adjusted to be constant at different column temperatures, the plots of $\ln V_N$ and $\ln V_R'$ versus $1/T$ should produce the same results for ΔH_{st}°. This variation of the hydrodynamic energy is a potential source of error in calculating thermodynamic quantities.

The material injected onto the column may not be ideal; but in cases where it is, we obtain

$$\gamma_s = \gamma_M = 1 \tag{12.35}$$

if it is ideal in both phases. If it is nonideal in one phase but ideal in the other, one of the activity coefficients must be unity.

Roles and Guiochon (19) developed a numerical method for determining the adsorption energy distribution function from adsorption isotherm data using gas–solid chromatography. They studied a variety of surface heterogeneous solids, such as aluminum oxide ceramic powders, which they coated on the

walls of open tubular columns. Using the classical method of elution but with larger volumes of organic vapors, they were able to calculate the distribution of adsorption energy of the probes on the surface.

12.3 SOLUTION THERMODYNAMICS

Although the chromatographic systems used for solution thermodynamic investigations generally have little analytical separations utility, the results do provide interesting evaluations of the interactions between solutes and solvents. In gas–liquid chromatography (GLC), one generally has the interaction of a "solvent" that is a liquid phase with a "solute" probe injected onto the column. Since only small quantities are injected onto the column, this situation approximates the conditions of infinite dilution in a solution. Retention results from the interaction occurring during dissolution of the "solute" in the "solvent." In this case, distribution of the solute between the mobile phase and the stationary phase results in equilibrium described by Raoult's law:

$$a_s^u = \frac{f}{f_0} = a_M^u \tag{12.36}$$

where f is the fugacity of the gas phase and f_0 is the standard-state fugacity. Replacing the fugacity by the activity coefficient multiplied by the activity coefficient γ_s, times the mole fraction X_s, one obtains

$$P = \frac{\gamma_s}{\gamma_M} X_s P^0 \tag{12.37}$$

Assuming that the gas phase is ideal ($\gamma_M = 1$) and remembering that this situation corresponds to infinite dilution ($\gamma_s = \gamma^\infty$), one obtains

$$P = \gamma^\infty X_s P^0 \tag{12.38}$$

The activity coefficient at infinite dilution accounts for deviations from the ideal form of Raoult's law. If the deviation is positive, $\gamma < 1$ and the solute's retention time is shorter than that of an ideal solute. If the deviation is negative, then $\gamma > 1$, and the retention time of the solute is increased. The adjusted retention volume and the pressure are related by the ideal-gas law:

$$P V_R' = n_s R T \tag{12.39}$$

This gives the following expression for the activity coefficient at infinite dilution ($n_s \gg N$, the number of solvent molecules):

$$\gamma^\infty = \frac{RT}{V_g M P^0} \tag{12.40}$$

TABLE 12.4 Sample Activity Coefficient Calculation

Data

$V_R = 600$ mL wt $= 2$ g $M = 114$ g/mol

$V_M = 100$ mL $T = 29^\circ$C $P^\circ = 140$ torr

Preliminary calculations

$$V_g = (V_R - V_M)/\text{wt} = (600\ \text{mL} - 100\ \text{mL})/2\ \text{g} = 250\ \text{mL/g}$$

$$T(\text{K}) = T(^\circ\text{C}) + 273.15 = 29 + 273.15 = 302.15\ K$$

$$P^\circ(\text{atm}) = P^\circ(\text{torr})/760\ \text{torr} = 140/760 = 0.184\ \text{atm}$$

$$R = 82.0573\ \text{mL atm}^{-1}\,\text{K}^{-1}\,\text{mol}^{-1}$$

$$\frac{RT}{V_g M P^\circ} = \frac{(82.0573\ \text{mL/atm K mol})(302.15\ \text{K})}{(250\ \text{mL/g})(114\ \text{g/mol})(0.184\ \text{atm})} = 4.73$$

where R = gas constant
T = absolute temperature
V_R' = adjusted retention volume
M = molecular weight of solvent
wt = weight of solvent on column
P° = vapor pressure of the pure solute

An example calculation is shown in Table 12.4.

The substituent constant of the Hammett equation has been related successfully to the logarithm of the activity coefficient ratio at infinite dilution for a series of *m*- and *p*-phenyl isomers. Hammett stated that a free-energy relationship should exist between the equilibrium or rate of behavior of a benzene derivative and a series of corresponding meta- and para-monosubstituted benzene derivatives. The Hammett equation may be written as

$$\log \frac{K_x'}{K_0'} = \sigma\rho \tag{12.41}$$

where K_x' (K_0') is the rate (or equilibrium) constant for a reaction of a substituted (unsubstituted) benzene derivative, respectively; σ is the Hammett substituent constant; and ρ is the Hammett reaction constant. The constant ρ may be used to establish a selectivity scale for stationary liquid phases in GLC through the relationship

$$\log \frac{\gamma_0^\infty}{\gamma_x^\infty} = \sigma_c\rho + b \tag{12.42}$$

where b is a constant. The chromatographic substituent constant σ_c is obtained from the following equation:

$$\sigma_c = 0.09 + 0.621 \log \frac{\gamma_0^\infty}{\gamma_x^\infty} \tag{12.43}$$

These relationships have been shown to predict the types of liquid phases necessary for optimum resolution.

Other important parameters measured by GLC are enthalpy, free energy, and entropy of solution. Although many—but by no means all—chemical reactions are produced at constant pressure and temperature in solution. The free-energy change of such a reaction determines whether it is spontaneous. Reactions with negative free-energy changes are spontaneous; those with positive free-energy changes are not. The dissolution process is important in this mechanism, and GLC can be used to measure thermodynamic properties for dissolution.

The enthalpy of solution is defined as the quantity of heat accompanying the following process:

$$\text{A (pure)} \rightarrow \text{A (dissolved in B)} \tag{12.44}$$

If the solution consists of two liquids, the resultant enthalpy change is sometimes called the *enthalpy of mixing.* The free energy and entropy of solution are analogously defined with respect to this process. Gas–liquid chromatography can be used to measure these thermodynamic parameters for a system consisting of a stationary phase that is the "solvent" B and where the "solute" A is injected onto the column. Under these conditions, the gas–liquid chromatographic retention measures this equilibrium between the solute and the dissolved solute. In general, one injects rather small quantities of A at very low dilution in B, that is, at infinite dilution. The free-energy change may be obtained directly from the partition coefficient K_D by Equation 12.15.

From Equation 12.30, one finds that each quantity may be considered to be divided into an "ideal" ($\gamma_s = \gamma_M = 1$) and a "nonideal" correction:

$$\overline{\Delta G^\circ} = \overline{\Delta G_i^\circ} + \overline{\Delta G_e^\circ} \tag{12.45}$$

$$\overline{\Delta H^\circ} = \overline{\Delta H_i^\circ} + \overline{\Delta H_e^\circ} \tag{12.46}$$

If the gas (or mobile) phase is considered to be ideal, the following GLC results obtain:

$$\overline{\Delta G_e^\circ} = -RT \ln \gamma^\infty \tag{12.47}$$

$$\overline{\Delta S_e^\circ} \frac{\overline{\Delta H_e^\circ} - \overline{\Delta G_e^\circ}}{T} \tag{12.48}$$

Another useful application of GC is measurement of the relative strengths of interaction of two solutes with a single stationary phase. Determination of the

differences in the free energies of the two components can be made readily from the chromatogram

$$\overline{\Delta G_2^\circ} - \overline{\Delta G_1^\circ} = -RT \ln \frac{t'_{R2}}{t'_{R1}} \tag{12.49}$$

where t'_{R1} is the adjusted retention time of substance 1 and the difference in the free energies is unaffected by flowrate, percent loading, or solvent molecular weight and may be used to study differences in interaction of isomers with a liquid phase or the comparison of isotopically substituted molecules.

Headspace GC takes advantage of the closed-vessel equilibrium between either a liquid or a solid and a gas. In the headspace analysis an aliquot of the equilibrated gas phase is removed from the vessel and the aliquot analyzed by GC. This technique is very convenient when direct sampling (solid or liquid) is difficult using traditional gas chromatographic techniques. In addition to the analysis application, headspace GC can also be used for determining physicochemical data (20). Labows (21) used automatic headspace GC to determine the solubilization behavior of volatile organic compounds in detergent surfactants. Since the sensory intensity and character of the flavor or fragrance depends on the solubility interactions in the system, it is important to quantify the interactions between the components and the surfactants used in the product. Using headspace GC, Labows determined the solubilization site within the micelle for a solute, the effect of the solute on the critical micelle concentration, the solute partition coefficient, and the effect of cosolvents on the critical micelle concentration.

Headspace GC can also be used to determine activity coefficients. Hussam and Carr (22) carried out a meticulous study that produced a device for automated measurements of both solute activity coefficients and vapor pressure (see also Section 12.4). The device allowed for rapid sample analysis, better than 0.01°C temperature control, the ability to work at low sample concentrations (mole fractions >0.01), minimal equilibrium perturbation by the sampling process, and the ability to vary solvent composition automatically.

12.4 VAPOR PRESSURE AND HENRY'S LAW

The equilibrium between a liquid and its vapor may be described by the *vapor pressure*, which is the amount of substance in the gas phase. The first accurate gas chromatographic thermodynamic measurements were of vapor pressure and enthalpies of vaporization made by Mackle et al. (23) using a gas chromatograph with a bypass sampling system. In this system the sample is placed in a sample tube and cooled with dry ice, and then is warmed to a specific temperature. After equilibrium is attained, the liquid sample is isolated from its vapor by a valve. The vapor is swept into a gas chromatograph by a carrier gas. The gas chromatographic detector is, in effect, measuring the vapor pressure at the temperature. Measurements for different temperatures give differing amounts, that is,

different vapor pressures. Plots of the logarithm of the peak area (or peak height for symmetric peaks) versus $1/T$ can be analyzed to obtain ΔH_v°, the enthalpy of vaporization from the slope. A rapid but approximate means of determining ΔH_v° is to extract it directly from a plot of the logarithm of the net retention volume versus the reciprocal of the column temperature. Table 12.5 gives some classes of compounds whose vapor pressures have been determined by GC.

The measurements made by Hussam and Carr were made via headspace GC and were limited by the detector's lower limit. Their technique is particularly valuable for low-volatility materials where conventional techniques are difficult because of the requirement that all gases and isomers of similar volatility be removed. Headspace GC circumvents those potential problems by removing interferences by the separation power of the chromatographic column.

A method for the rapid determination of relative vapor pressure by capillary GC was reported by Westcott and Biddleman (24), based on comparison of the adjusted retention volume of the sample to that of a substance (V'_{R2}) whose vapor pressure at the column temperature is known to be P_2° (25). The adjusted retention volumes are related to the known vapor pressures:

$$\ln \frac{V'_{R1}}{V'_{R2}} = A \ln P_2^\circ + C \tag{12.50}$$

TABLE 12.5 Vapor Pressures of Compounds Determined by GC

Compound	Reference
Inorganic chlorides and oxychlorides	[a]
Propanol, butanol, 2-butanone, 3-butanone, *n*-heptane, *p*-dioxane	[b]
Benzene, toluene, butyl acetate	[c]
Metal carbonyls	[d]
Fatty acids, fatty esters, fatty alcohols, chloroalkanes	[e]
Alcohols	[f]
Perfumes	[g]
Hydrogen saturated with methanol	[h,i]
Pentane, hexane, heptane, octane, benzene, toluene, nitromethane, acetonitrile, methylethylketone, dioxane, ethanol	[j]

[a] S. Se, J. Bleumer, and G. Rijnders, *Sep. Sci.* **1**, 41 (1966).
[b] G. Geisler and R. Janash, *Z. Phys. Chem.* **233**, 42 (1966).
[c] A. Franck, H. Orth, D. Bilinmaier, and R. Nussbaum, *Chem. Z. Chem. Appl.* **95**, 219 (1971).
[d] C. Pommier and G. Guiochon, *J. Chromatogr. Sci.* **8**, 486 (1970).
[e] A. Rose and V. Schrodt, *J. Chem. Eng. Data* **8**, 9 (1963).
[f] F. Ratkovics, *Acta Chim. Acad. Sci. Hung.* **49**, 57 (1966); through *G. C. Abstr.* 442 (1967).
[g] S. A. Voitkevich, M. M. Schedrina, and N. P. Soloveva, *Rudol'fi Maslo-Zh., Prom.* **37** 27 (1971); through *Chem. Abstr.* **76**, 251 (1972).
[h] F. Ratkovics, *Acta Chim. Acad. Sci. Hung.* **48**, 71 (1966); through *G. C. Abstr.* 443 (1967).
[i] F. Ratkovics, *Magyar Kem. Foluoirat* **72**, 186 (1966); through *Chem. Abstr.* **65**, 1450 (1966).
[j] A. Hussam and P. W. Carr, *Anal. Chem.* **57**, 793 (1966).

where A and C are constants, if the enthalpies of vaporization of the two components are assumed constant. Thus A and C are determined from the slope and intercept of the straight-line plot of $\ln (V'_{R1}/V'_{R2})$ versus $\ln P_2^\circ$. Knowledge of these constants allows one to calculate the vapor pressure of the test material P_1°:

$$\ln P_1^\circ = (1 - A) \ln P_2^\circ - C \tag{12.51}$$

This method is useful for narrow temperature ranges ($<70^\circ$C) and when the reference and test materials are similar, with the vapor pressures of the reference material obtained from other techniques (26).

Henry's law describes the distribution of a discrete quantity of solute between a dilute solution and the gas phase above it. The Henry's law constant K_H is the ratio of a compound's abundance in the gas phase to that in the aqueous phase at equilibrium (27). K_H can be estimated from vapor pressure and solubility data. Calculated values seldom agree with those measured experimentally, however, so GC can be used to obtain K_H data readily.

McAuliffe developed a novel method using a 50-mL gastight syringe for gas chromatographic determination (28). The method is based on the analysis of a single phase after two successive phase equilibrations using equal volumes of gas and liquid phases. The Henry law constants for hydrocarbons with up to 10 carbon atoms were determined successfully.

Miller and Stuart devised a method that combined an equilibrium vessel made of a large stopcock and a static headspace method to measure K_H (29). Using the phase ratio obtained from the experimental apparatus and plotting (versus the initial peak area of the analyte) a series of peak areas of the analyte in the headspace after equilibrium, they were able to obtain K_H using only one aqueous solution (even if its concentration is unknown).

12.5 COMPLEXATION CONSTANTS

Metal ions can act as electron pair acceptors, reacting with electron donors (ligands) to form coordination compounds or complexes. The ligand must have at least one pair of unshared electrons with which to form the bond. Chelates are a special class of coordination compound, resulting from the reaction of a metal ion with a ligand having two or more donor groups.

Complexes often form in steps, with one ligand added in each step:

$$\mathrm{M} + \mathrm{L} \overset{k_1}{\leftrightarrow} \mathrm{ML}$$

$$\mathrm{ML} + \mathrm{L} \overset{k_2}{\leftrightarrow} \mathrm{ML_2}$$

$$\mathrm{ML}_{n-1} + \mathrm{L} \overset{k_n}{\leftrightarrow} \mathrm{ML}_n$$

The stepwise constants for the equilibria specified by this sequence of reactions are called the *formation* or *stability constants*:

$$k_1 = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \tag{12.52}$$

$$k_n = \frac{[\mathrm{ML}_n]}{[\mathrm{ML}_{n-1}][\mathrm{L}]} \tag{12.53}$$

The more stable a complex, the larger is its formation constant. The reciprocal of the formation constant is the instability constant. Determination of these formation constants is relevant to understanding the chemistry of such systems. Like many other equilibrium constants, such stability constants may be determined by gas chromatography.

Purnell (30) surveyed numerous gas chromatographic approaches to the study of complex equilibria and developed generalized retention theories for each kind. His classification system, which is summarized in Table 12.6, greatly simplifies the approach to complexation reactions.

The important point is that, if the gas chromatograph is set up in such a way that the retention is dominated by the interaction of the ligand with the electron acceptor, the gas chromatographic retention data can be used to measure the stability constant of the complex. As an example, consider the determination of formation constants of complexes of aromatic electron donors and di(*n*-propyl)tetrachlorophthalate in an inert solvent [class A(II)]. If a one-to-one complex is assumed, the reactions occurring on the column are

$$\mathrm{A\ (gas)} \overset{K_\mathrm{R}^0}{\leftrightarrow} \mathrm{A\ (S)} \tag{12.54}$$

$$\mathrm{A\ (S) + D\ (S)} \overset{K_1}{\leftrightarrow} \mathrm{AD\ (S)} \tag{12.55}$$

where A is volatile di(*n*-propyl)tetrachlorophthalate, D is a nonvolatile electron donor in the stationary phase, S is the solvent phase (D + I), I is a nonvolatile inert solvent in which D is dissolved, K_R° is the distribution constant (partition coefficient) of uncomplexed A between S and the gas phase, and K_1 is the formation constant of AD in solution. If AD(S) and A(S) are assumed to approach

TABLE 12.6 Purnell Classification System for Complexes

Class	Type I	Type II	Type III
A	AX_n	XA_m	A_mX_n
B	SX_n	XS_p	S_pX_n
C	X polymerizes in solution	X depolymerizes in solution	
D	$SA_{m,m+1\ldots}$	$AS_{p,p+1\ldots}$	

Key: A, additive; X, solute; S, solvent; $n \leq 1$, $m \leq 1$, $p \leq 1$.

infinite dilution (a reasonable assumption in a gas chromatographic system), the activity coefficients approach 1, and

$$K_1 = \frac{[\mathrm{AD}]}{[\mathrm{A}]a_\mathrm{D}} \tag{12.56}$$

where a_D is the activity of the donor in the stationary phase. The apparent gas chromatographic distribution constant, assuming ideal solution, is

$$(K_\mathrm{R})_\mathrm{S} = K_\mathrm{R}^0(1 + K_1[\mathrm{D}]) \tag{12.57}$$

where [D] is the concentration of electron donor in the stationary phase. This constant is related to the corrected net retention volume V_N *by*

$$V_\mathrm{N} = (K_\mathrm{R})_\mathrm{S} V_\mathrm{S} \tag{12.58}$$

where V_S is the total solvent volume. Determination of the gas chromatographic retention volume of the acceptor on columns containing varying amounts of the donor allows determination of the formation constant. From Equation 12.57 it is clear that a plot of $(K_\mathrm{R})_\mathrm{S}$ versus concentration [D] will be linear with an intercept K_R^0 and a slope of $K_1 K_\mathrm{R}^0$.

Martire and co-workers (31,32) developed a method of determining complexation constants that is much less time-consuming than the Cadogan–Purnell method (33) but makes additional assumptions. They have demonstrated that the specific retention volume of A is related to the complex formation constant:

$$(V_\mathrm{g}^0)_\mathrm{D} = \frac{273R(1 + K_1 a_\mathrm{D})}{\gamma_\mathrm{A}^\mathrm{D} P_\mathrm{A}^\circ M_\mathrm{D}} \tag{12.59}$$

where $(V_\mathrm{g}^0)_\mathrm{D}$ is the specific retention volume of A in a system of pure D, R is the gas constant, $\gamma_\mathrm{A}^\mathrm{D}$ is the activity coefficient of uncomplexed A in D at infinite dilution, P_A° is the vapor pressure of A, and M_D is the molecular weight of D. In the Martire–Riedl method (31), only two columns are used, one containing D and the other containing a reference liquid phase R of approximately the same molecular size, shape, and polarizability as D [class B(II)]. Utilizing a noncomplexing solute N on R and D, one may measure all the various specific retention times.

Assuming

$$\frac{\gamma_\mathrm{N}^\mathrm{R}}{\gamma_\mathrm{N}^\mathrm{D}} \approx \frac{\gamma_\mathrm{A}^\mathrm{R}}{\gamma_\mathrm{A}^\mathrm{D}} \tag{12.60}$$

(which occurs because of the assumed similarity of R and D), one obtains an equation of the form

$$\frac{(V_\mathrm{g}^0)_\mathrm{D}^\mathrm{A}(V_g^0)_\mathrm{R}^\mathrm{N}}{(V_\mathrm{g}^0)_\mathrm{R}^\mathrm{A}(V_\mathrm{g}^0)_\mathrm{D}^\mathrm{N}} = 1 + K_1 a_\mathrm{D} \tag{12.61}$$

TABLE 12.7 Comparison of Cadogan–Purnell (C/P) and Martire–Riedl (M/R) Methods of Complex Formation Constant Determination

		Complex Formation Constant at 40°C		
Electron Donor	Electron Acceptor	C/P Method	M/R	Method
Di-*n*-octylamine	$CHCl_3$	0.405 ± 0.19[b]	0.403[a]	0.392[c]
Di-*n*-octylamine	CH_2Cl_2	0.179 ± 0.014	0.187	0.181
Di-*n*-octylamine	CH_2Br_2	0.222 ± 0.004	0.219	0.224

[a] From Reference 24.
[b] From Reference 23.
[c] From J. P. Sheridan, D. E. Martire, and F. P. Banka, *J. Am. Chem. Soc.* **95**, 4788 (1973).
Source: From Reference 24. Used with permission from *Anal. Chem.* **45**, 2087 (1973). Copyright American Chemical Society.

where, generally, $(V_g^0)_j^i$ is the specific retention volume of i on a column of j. Liao et al. (34) used the Martire–Riedl and the Cadogan–Purnell methods for three-electron donor systems to make a comparison. As Table 12.7 shows, the two methods are in excellent agreement.

A theoretical study to compare the methods of GC and mass spectrometry for measuring weak complexation constants has been performed by Eon and Guiochon (35), who showed that both methods lead to the same results if properly used. Discrepancies can usually be attributed to misinterpretation of the chromatographic measurement.

12.6 VIRIAL COEFFICIENTS

The mathematical relationship between pressure, volume, temperature, and the number of moles of gas at equilibrium is given by its equation of state. The best-known equation of state is the ideal-gas law, $P\overline{V} = RT$, where P is the pressure of the gas, $\overline{V}$ is its molar volume (V/n), n is the number of moles of gas, R is the gas constant, and T is the absolute temperature of the gas. Many modifications of the ideal-gas equation of state have been proposed so that the equation can fit P, V, T data of real gases. One of these equations, the virial equation of state, accounts for nonideality by utilizing a power series in the density

$$\frac{P\overline{V}}{RT} = 1 + B\rho + C\rho^2 + D\rho^3 + \cdots \tag{12.62}$$

where $\rho = 1/\overline{V}$ and B, C, and D are the second, third, and fourth on virial coefficients, respectively. The values of the virial coefficients are functions of temperature and the particular gas under consideration but are independent of density and pressure.

12.6.1 Second Virial Coefficients

The virial equation of state is especially important since its coefficients can be modeled in terms of nonideality resulting from interactions between molecules. Thus a link is formed between macroscopic gas properties (P,V,T) and the forces between molecules. For a multicomponent mixture, multiple second virial coefficients are needed, one to account for each pairwise interaction. The second virial coefficients for a two-component mixture are B_{11}, B_{12}, and B_{22}, where B_{11} represents the interaction between two molecules of component 1, B_{12} represents the interaction between a molecule of 1 and a molecule of 2, and B_{22} represents interaction between two molecules of 2. A tabulation of some compounds whose virial coefficients have been measured by GC is given in Table 12.8.

The method of determining virial coefficients by GC consists of measuring the retention volumes at various carrier-gas pressures and extrapolating to zero pressure. Three extrapolation procedures have been suggested, although they do not give the same results. The method due to Cruickshank et al. (36), which takes into account carrier-gas flowrate and local pressure, is most promising.

The least complicated equation for determination of B_{12} is given by Cruickshank et al. (37)

$$\ln V_N = \ln V_N^0 + \beta P_o J_3^4 \tag{12.63}$$

where V_N is the net retention volume; P_o is the outlet pressure; $\beta = (2B_{12} - V_1)/RT$; V_1 is the partial molar volume of the sample at infinite dilution in the stationary (liquid) phase; 1 refers to the sample component, 2 to the carrier gas,

TABLE 12.8 Second Virial Coefficients Measurements by Gas Chromatography

Compounds	Reference
H_2, N_2, O_2, CO_2, hydrocarbons	*a*
Hydrocarbons	*b*,*c*,*d*
Hydrocarbons, permanent gases	*e*
Benzene–gas mixtures	*f*
Benzene–CO_2, benzene–N_2	*g*
Higher hydrocarbons and their derivatives	*h*
Benzene–N_2, cyclohexane, *n*-hexane, diisodecylphthalate	*i*

[a] D. H. Desty, A. Goldup, G. Luckhurst, and W. Swanton, in *Gas Chromatography*, M. von Sway, ed., Butterworths, London, 1962.
[b] L. Che Kalov and K. Porter, *Chem. Eng. Sci.* **22**, 897 (1962).
[c] E. M. Dentzler, C. Krobles, and M. L. Windsor, *J.* Chromatogr. **32**, 433 (1968).
[d] R. L. Pecsok and M. L. Windsor, *Anal. Chem.* **40**, 1238 (1968).
[e] P. Chavin, *Bull. Soc. Chim. Fr.*, 1964, 1800; through *G. C. Abstr.* 965 (1966).
[f] C. R. Coan and A. D. King, *J. Chromatogr.* **44**, 429 (1969).
[g] A. J. B. Cruickshank, B. W. Gainey, C. P. Hicks, T. M. Letcher, R. W. Moody, and C. L. Young, *Trans. Farad. Soc.* **65**, 105 (1969).
[h] M. Vidergauz and V. Semkin, *Zh. Fiz. Khim.* **45**, 931 (1971).
[i] B. K. Raul, A. P. Kudchadker, and D. Devaprabhakova, *Trans. Farad. Soc.* **69**, 1821 (1969).

and 3 to the stationary liquid; and J_3^4 is a function of the column inlet and outlet pressures P_i and P_o:

$$J_n^m = \frac{n}{m}\left[\frac{(P_i/P_o)^{m-1}}{(P_i/P_o)^{n-1}}\right] \tag{12.64}$$

B_{12} is obtained from the slope of a plot of ln V_N versus $P_0 J_3^4$.

12.6.2 Gas–Solid Virial Coefficients

Determination of gas–solid virial coefficients can be useful in explaining the interaction between an adsorbed gas and solid surface. The terms are defined so that the number of adsorbate molecules interacting can be readily ascertained. For example, the second-order gas–solid interaction involves one adsorbate molecule and the solid surface; the third-order gas–solid interaction involves two adsorbate molecules and the surface, and so on. The number of adsorbed molecules under consideration is expanded in a power series with respect to the density of the adsorbed phase, as is done for a bulk gas.

Few determinations of gas–solid virial coefficients have been made. Halsey and co-workers (38,39) used the temperature dependence of the first gas–solid virial coefficient to calculate the potential energy curve for a single molecule in the presence of a solid. Hanlan and Freeman (40) showed that this coefficient may be obtained from frontal analysis of gas chromatographic data. Rudzinski et al. (41,42) first used the second and third gas–solid virial coefficients obtained from gas chromatographic data to estimate surface areas. The surface area of silicagel determined by use of virial data was greater than that obtained from the BET method. The discrepancy was explained by noting that the BET method does not take the lateral interactions into account. These interactions decrease the effective area of the adsorbent, thus making the calculated BET area less than it should be.

12.7 KINETICS

Chemical kinetics describes the progress of a chemical reaction. The most common description of the progress is given by the term "rate of reaction"—a positive quantity that expresses how the concentration of a reactant or product changes with time. For the elementary reaction

$$\mathrm{A} \rightarrow \mathrm{P}$$

the rate is proportional to the appearance of product per unit time or the disappearance of reactant per unit time:

$$\begin{aligned} \text{Rate} &\propto \frac{d[\mathrm{P}]}{dt} \\ \text{Rate} &\propto -\frac{d[\mathrm{A}]}{dt} \end{aligned} \tag{12.65}$$

Gas chromatography is a versatile tool for studying many reactions, especially in multicomponent systems, process reaction studies, or catalytic reactions. Samples can be taken from a reaction mixture at different time intervals and chromatographed. The rate is calculated from these concentration changes as a function of time. Alternatively, the chromatograph can be interfaced directly to the reactor where kinetic studies are performed directly (43).

The chromatographic column has been used as a reactor to study kinetics of dissociation. The reactant is introduced as a pulse at the head of the column and is continuously converted to product and separated as it travels through the column. The apparent rate constant (k_{app}) is a function of the rate of the liquid (stationary)-phase reaction (k_1), the rate of the gas (mobile)-phase reaction (k_g), the residence time in the gas phase (t_g), and the residence time in the liquid phase (t_1):

$$k_{app} = k_1 + \left(\frac{t_g}{t_1}\right) k_g \tag{12.66}$$

A mathematical statement of the dependence of the rate on the concentrations of components is called the *rate equation*; for example, rate $= k$[A][B], where k is the rate constant and [A] and [B] represent the concentrations of reactants. From the rate equation one can frequently extract information on the mechanism (i.e., the path followed to convert reactants to products). Improvements in the gas chromatographic measurement of kinetic data have followed improvements in microchemical techniques and improvements in gas chromatographic instrumentation. Bertsch et al. (44) showed how microscale techniques can be applied to online reaction GC. They developed techniques at the nanogram scale for both online and postcolumn reactions.

Economopoulos et al. (45) used reversed-flow GC to study the kinetics of alcohol fermentation. In reversed-flow GC, extra chromatographic sample peaks are created by reversing the carrier-gas flow direction for short time intervals during the course of the experiment. These narrow extra peaks are superimposed on normal elution curves to elicit information that is used in conjunction with measurements of suspended particles in the fermenting medium. The reversed-flow technique reduces two potential sources of error: the impact from lengthy high-temperature interactions on the column and the minimization of interaction with the gas chromatographic packing material. Both of these potential sources of error could alter the composition of the sample in this complex biosystem.

Chai et al. (46) developed a novel automated gas chromatographic technique to study slow kinetic processes. The technique uses multiple headspace extraction and can be applied to reactions involving volatile formation or adsorption/desorption phenomena.

Activation energies may be derived from gas chromatographic data. The activation energy describes how temperature affects chemical reaction rate. Unless the thermal energy RT is near the activation energy (or greater), the rate constant will not be near its maximum value. A plot of ln k versus $1/T$ is linear and the slope is $-E_a/R$, where E_a is the activation energy and R is the gas constant

(1.987 cal K^{-1} mol^{-1}). A useful expression relating the rate constants k_2 and k_1 at two different temperatures T_2 and T_1 is

$$\ln \frac{k_2}{k_1} = -\frac{E_a}{R}\left(\frac{T_2 - T_1}{T_1 T_2}\right) \tag{12.67}$$

Therefore, if E_a and k_1 are known, a rate constant for any temperature may be calculated.

12.8 PYROLYSIS, THERMOLYSIS, AND COMBUSTION

Pyrolysis, thermolysis, and combustion techniques are often used in conjunction with gas chromatography. *Pyrolysis* is chemical degradation caused by input of thermal energy. Generally pyrolysis is performed in an inert (not reductive or oxidative) environment. *Thermolysis* is the uncatalyzed cleavage of chemical bonds resulting from the exposure of a compound to high temperature. *Combustion* is an exothermic reaction in which oxidation occurs. Combustion usually requires oxygen (as in air).

Pyrolysis gas chromatography (PGC) was one of the first combination gas chromatographic techniques, yet it is still plagued by problems of accuracy and repeatability of pyrolysis conditions and laboratory-to-laboratory reproducibility. There are three major devices for PGC: (1) heated wire or ribbon, (2) tube furnace, and (3) Curie point filament. The heated wire or ribbon apparatus uses resistive heating to provide flash pyrolysis from ambient temperature to 1400°C. It can be controlled to reach the maximum temperature in milliseconds or at some fixed rate, and the device can hold the top temperature for a settable fixed time. These high-precision devices can be placed directly in the injection port (for vertical injection ports). The temperature reading should be checked from time to time to ensure accuracy.

The classical tube furnace is the oldest and simplest device for implementing PGC. A sample is placed in a boat (e.g., quartz or platinum), and the boat is placed in a quartz tube. The furnace is moved over the sample, or the sample boat is pushed into the furnace. Carrier gas is swept through the tube, and the pyrolysis products are swept into the chromatograph through a sampling valve. This method suffers from reproducibility of sample introduction and temperature lag. The chromatographic peak shape is generally broader than in other PGC methods. Newer microfurnace reactors give products that can be directly analyzed via online high-resolution GC (47).

Curie point pyrolysis involves coating of the sample on a ferromagnetic conductor (wire or capillary tube). The conductor is inductively heated to a specific temperature when exposed to a radiofrequency field. The composition of the conductor determines the Curie temperature (300–1000°C). The major advantage of the Curie point PGC is the ability to heat samples reproducibly to accurately defined temperatures in milliseconds. The major disadvantage is the inability to vary temperature since a different rod is needed for each point.

By far the largest, most successful applications of PGC have been to the characterization of synthetic polymer microstructure. PGCs of these compounds yield information such as monomer identity and content, purity, and presence of additives. PGC is even more powerful for solving these types of problems when coupled with spectroscopic detectors. For example, Sahota et al. (48) showed that single-step PGC coupled with mass spectrometry could be used to measure the DNA content of cultured mammalian cells.

12.9 OTHER APPLICATIONS OF GC TO PHYSICOCHEMICAL MEASUREMENTS

12.9.1 Catalysis

Gas chromatography has been used to measure catalyst diffusivities, surface area, active surface area, kinetics, thermodynamics of adsorption, and pore size distribution and to study mechanisms and follow catalyst performance. Some examples are given in Table 12.9.

12.9.2 Photochemistry

Gas chromatography may be used to separate photochemically derived species either on or off line. If one uses a glass or quartz column or vaporizer, the study

TABLE 12.9 Applications of Gas Chromatography to Catalysis

Application	Reference
Acidity and catalytic selectivity in the Na–H–mordenite system	[a]
Displacement of H_2 from Rh surface by CO	[b]
Environmental catalysis studied by reversed-flow gas chromatography	[c]
Hydrogenation of olefins on a Pt/Ir GC/MS interface	[d]
Destruction of polychlorodibenzene-*p*-dioxins	[e]
Solid–liquid phase transfer catalysis—carboxylic acid alkylation	[f]
Thiophene poisoning of copper chromite	[g]
Thermoprogrammed reduction of cobalt oxide catalysts	[h]
Hydrodenitrogenation catalysis by reversed-flow GC	[i]

[a] P. Ratnasamy, S. Sivashkar, and S. Vishmoi, *J. Catal.* **69**, 428 (1981).
[b] W. K. Jozwiak and T. Paryjczak, *React. Kinet. Catal. Lett.*, **18**, 163 (1981).
[c] A. Kalantzopoulos, Ch. Abatzoglou, and F. Roubani-Kalantzopoulou, *Colloids Surf. A: Physicochem. Eng. Aspects* **151**, 377 (1999).
[d] G. C. Jamieson, *J. High Resol. Chromatogr. Chromatogr. Commun.* **5**, 632 (1982).
[e] D. C. Ayres, *Nature* **290**, 323 (1981).
[f] A. Arbin, H. Brink, and J. Vesiman, *J. Chromatogr.* **196**, 255 (1980).
[g] V. R. Choudhary and S. D. Sansare, *J. Chromatogr.* **192**, 420 (1980).
[h] T. Paryjczak, J. Rynkowski, and S. Karski, *J. Chromatogr.* **188**, 254 (1980).
[i] A. Niotis and N. A. Katsanos, *Chromatographia* **34**, 398 (1992).

TABLE 12.10 Applications of Gas Chromatography in Photochemistry

Application	Reference
Photochemistry within a glass gas chromatographic column	[a]
Photodecomposition of sulfonamides and tetracyclines	[b]
Photoreduction of methylviologen adsorbed on cellulose	[c]
Radiolysis of D,L-tryptophan	[d]
Photolysis of dichlorofluanid	[e]

[a] W. G. Laster, J. B. Pawliszyn, and J. B. Phillips, *J. Chromatogr. Sci.* **20**, 278 (1982).
[b] W. H. K. Sanniez and N. P. Ipel, *J. Pharm. Sci.* **69**, 5 (1980).
[c] M. Kaneko, J. Motoyoshi, and Y. Yamada, *Nature* **285**, 1468 (1980).
[d] W. A. Bonner, N. E. Balir, and J. J. Flores, *Nature* **281**, 150 (1979).
[e] T. Clark and D. A. M. Watkins, *Pest. Sci.* **9**, 225 (1978).

might be done on the column itself. Table 12.10 gives some examples of GC used in photochemistry.

12.9.3 Inverse Gas Chromatography

Inverse gas chromatography (IGC) is different from conventional gas chromatography in that the stationary phase is the analyte. The mobile phase is used to convey probes of known characteristics. The output from the experiment (retention time, peak shape, etc.) is monitored to glean information about the stationary phase. The stationary phase may be composed of fibers, polymer pellets, minerals, or a substance coated on an inert chromatographic support of the wall of the chromatographic column (49). IGC has been especially useful for characterizing polymeric species (50). Measurements such as degree of crystallinity, glass and melting transition temperatures, solubility parameters, diffusion properties, interaction parameters with polymer blends, and interfacial and surface properties have been carried out on a variety of systems. Table 12.11 gives a variety of examples.

12.9.4 Simulated Distillation

Gas chromatographic retention data can be used to simulate the results of fractional distillation. The separation takes place on a chromatographic column whose interactions (between sample components and the stationary phase) give a sample elution profile that can be correlated with its boiling point distribution. Boiling range distribution profiles are especially important in the petroleum industry, where such information may be used to control refining operations and specifications testing, to determine the commercial value of crude oil to a refiner, to calculate vapor pressure of gasoline or gasoline fractions (used to describe automobile performance parameters), or as a "fingerprint" (51) to help identify the source of a spill or leaking underground storage tank. The chromatographic procedure is often called *SIMDIS* for "simulated distillation" analysis.

TABLE 12.11 Applications of Inverse Gas Chromatography

Application	Reference
Cotton fabrics	[a]
Interactions between solvents and linear or branched polystyrene	[b]
Column preparation	[c]
Organic solute solubility in polymer films	[d]
Surface properties of active carbons	[e]
Interaction of polyetherpolyurethane with solvents and solubility parameter	[f]
Polystyrene–hydrocarbon interaction parameters and solubility parameter	[g]
Polycarbonate surface energies and interaction characteristics	[h]
Surface heterogeneity of alumina oxide ceramic powders	[i,j]
Surface energy distribution	[k]

[a] E. Cantergiani and D. Benczedi, *J. Chromatog. A* **969**, 103 (2002).
[b] M. Galin, *Polymer* **19**, 596 (1978).
[c] T. Inui, Y. Marakami, T. Suzuki, and Y. Takegami, *Polym. J* (Tokyo) **14**, 261 (1982); through *Chem. Abstr.* **97**, 39599e (1982).
[d] J. E. G. Lipson and J. E. Guillet, *J. Coat. Technol.* **54**, 89 (1982).
[e] F. J. Lopez-Garzon, M. Pyda, and M. Domingo-Garcia, *Langmuir* **9**, 531 (1992).
[f] A. M. Faarooque and D. D. Deshpande, *Eur. Polym. J.* **28**, 1547 (1992).
[g] E. Ozdemir, A. Acikses, and M. Coskun, *Macromol. Rep.* **A29**, 63 (1993).
[h] U. Panzer and H. P. Schreiber, *Macromolecules* **25**, 3633 (1992).
[i] J. L. Roles and G. Guiochon, *J. Chromatogr.* **233**, 591 (1992).
[j] J. L. Roles and G. Guiochon, *Anal. Chem.* **64**, 25 (1992).
[k] J. L. Roles and G. Guiochon, *J. Phys. Chem.* **95**, 40098 (1991).

In SIMDIS the sample is introduced onto a chromatographic column that separates components in boiling point order. The column temperature is programmed up and the area under the chromatogram recorded. When a suitable calibration mixture is used, the retention time axis may be correlated directly with boiling temperature to produce a graph of the amount of material boiling at a specific temperature. From the boiling temperature and the chromatographic areas, the boiling range distribution of the sample is obtained.

Two gas chromatographic methods are designated by the American Society for Testing and Materials, ASTM. ASTM method D2887 (52) is used for determining the boiling range distribution of petroleum fractions with a final boiling point of 538°C or lower. ASTM method D3710 (53) is used for determining the boiling range distribution of gasoline and gasoline fractions with a final boiling point of 260°C or lower. Method D3710 is sometimes referred to as *gas chromatographic distillation* (GCD). Both methods also recommend how to prepare calibration standards for SIMDIS.

Since neither ASTM method makes specific recommendations regarding the gas chromatographic column, any column can be used that meets the method's specifications for separation in order of boiling point and certain column performance requirements regarding resolution, system noise and sensitivity, drift, and so on. Consequently, high-resolution GC has been increasingly applied to

SIMDIS analyses (54–57), since most commercial high-resolution columns can surpass the recommended column performance characteristics.

12.9.5 Solubility

Knowledge of the partitioning of substances into various environmental compartments is essential to understanding environmental fate, effects, and methods for remediation. Common measurement techniques such as ASTM E1148-87 (58) suggest methods for preparing the solubility samples, but seldom specify the analytical measurement technique. As environmental scientists become interested in materials with low solubility (ppb, ppt), the only feasible technique that gives both compositional and concentration data are gas chromatography mass spectrometry or liquid chromatography mass spectrometry.

12.10 QUALITY ASSURANCE, ACCURACY, PRECISION, AND CALIBRATION

The accuracy and precision of physicochemical measurements by GC rely on the ability of the gas chromatograph to control and measure all parameters related to the required chromatographic data. Temperature, in general, should be controlled and known to at least $\pm 0.005^{\circ}$C. In some cases the stationary phase mass must be known within $\pm 0.2\%$.

Most chromatograph manufacturers claim that temperature can be controlled within 0.25°C on modern chromatographic instruments. That does not mean that the temperature is accurate within the range, however. Furthermore, we have seen that temperature gradients exist within the oven and even within the column itself. For most physicochemical measurements, therefore, the instrumentation must be tested to determine the precision and accuracy of the essential settings.

It is not easy to determine which factors play the greatest role in obtaining good accuracy and precision. One must consider the assumptions inherent in the theory as well as the chemical, mechanical, and instrumental parameters. In general, gas chromatographic methods agree within 1–5% with other physicochemical methods. For example, Hussam and Carr (22) showed that in the measurement of vapor/liquid equilibria via headspace GC, complex thermodynamic and analytical correction factors were needed. These often came from other experimental measurements that were not necessarily accurately known. Another source of significant error can be in determination of the mass of stationary phase contained within the column (59). Other sources of error include measurement of holdup time (60), flowrate, sample mass, response factors, peak area, or baseline fidelity.

The general issues concerning chromatographic calibration have been addressed in Chapter 7. In the measurement of physicochemical properties by GC, one must also address how the data were obtained on the “known” species. This usually entails some careful literature inspection, including determining the mechanism and underlying principles used in the method. Another important

factor in making relative measurements is how closely the model compound resembles the "unknown." In any event, the chromatographer needs to consider all possible types of interactions that might occur from sample preparation through detection. Isotopically labeled internal standards and surrogates can be just as useful in the measurement of physicochemical properties as they are in "traditional" quantitative analysis. With all these caveats, however, the speed and simplicity of the gas chromatographic method should continue to make it very attractive for making physicochemical measurements.

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CHAPTER THIRTEEN

Petroleum and Petrochemical Analysis by Gas Chromatography

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13.5.2 Typical Applications
ACKNOWLEDGMENTS
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13.1 INTRODUCTION

Gas chromatography has been developed into a key analytical tool for the petroleum and petrochemical industry. This chapter serves as an introduction to the application of gas chromatography in this field. For petroleum, it covers the exploration, production, and refining of crude oil. Applications for the major derivatives of petroleum that are basic to the chemical industry are discussed in Section 13.4. Process gas chromatography is also discussed. In addition to the routine analyses, an attempt has been made to indicate the potential for the further development of gas chromatography in the petroleum industry. For a more detailed review of chromatography in the petroleum industry, the reader is referred to the books edited by K. H. Altgelt et al. (1,2). Also, a general review of the chemistry and technology of petroleum and petrochemicals has been provided by Speight (3–6) and Mator and Hatch (7).

13.1.1 Historical Perspective

The analysis of hydrocarbons in petroleum and its products began in the mid 1800s. The original methods were based on physical properties such as boiling point and specific gravity. In 1928, the American Petroleum Institute (API) initiated Project 6 to separate, identify, and determine the chemical constituents of commercial petroleum fractions. From this program, column adsorption chromatography was developed for separating components by hydrocarbon type. Because of the military needs of World War II in the 1940s, petroleum laboratories quickly developed spectroscopic methods. Mass spectrometry was introduced in 1943 for gas analysis. It then began to replace the low-temperature fractional distillation method for light hydrocarbons that was developed by Podbielniak (8). Then in the early 1950s, gas chromatography (GC) was developed.

The development of GC and the analysis of petroleum and petrochemicals have enjoyed a mutually beneficial relationship. Indeed, the first international symposium on vapor-phase (gas) chromatography was sponsored by the British Institute of Petroleum in 1956 (9). Papers describing the analysis of refinery gas, solvents, aromatics in coal-tar naphthas, and samples from the internal combustion engine were presented. Most of the work included in these presentations was done on “homebuilt” chromatographs. The first commercial gas chromatograph or “vapor-phase fractometer” was also described, along with the first ionization detector.

The rapid development of GC continued to parallel the refinement of petroleum applications. Eggertsen and his co-workers (10) in 1956 described a 50-ft column

packed with carbon black containing 1.5% squalene to separate 10 major C5–C6 saturates in 2 h. By 1958 the same workers used other column packings and extended the analysis of the saturates to C7. All but two of the 24 C6–C7 saturates were resolved in 12–16 h.

The second international symposium was held in 1958 and was again sponsored by the British Institute of Petroleum (11). Improved techniques allowed Scott (11) to separate the C7 and C8 paraffin isomers, using a column with 30,000 theoretical plates. Golay (12) also described the potential of open tubular capillary columns. These highly efficient columns were readily adopted by the petroleum industry. In 1961, Desty et al. (13) reported on the use of a 900-ft glass open tubular column coated with squalene to resolve 122 peaks from C3 to C9 in 20 h. This work was part of the API Project 6. In 1968, Sanders and Maynard (14) published a method for C3–C12 hydrocarbons in gasoline. They performed this analysis in less than 2 h on a 200-ft squalene column by using both flow and temperature programming.

The trend has been toward the increased use of GC in the petroleum industry. This is due to the relatively low cost of a gas chromatographic system that can provide more detailed analyses as well as the detection of trace components. Gas chromatography has also provided a means for online process monitoring. Future utilization of GC will involve the establishment of basic relationships between composition and performance parameters. This will allow the substitution of this rapid and reliable chromatographic analysis for the empirical methods from the past.

13.1.2 Standardization of Analyses

The concept of standardized testing is important in the petroleum industry, as it is in many others. Its usefulness for commodity-type products that are widely bought, sold, and exchanged is apparent. The American Society for Testing and Materials (ASTM) has provided these standardized procedures that are required for product specification testing.

The ASTM is an international, nonprofit, technical, scientific, and educational society that was formed in 1898. Its purpose is the development of standards on the characteristics and performance of materials, products, systems, and services and the promotion of related knowledge. Because of the support of the petroleum industry, the ASTM represents a source of voluntary consensus standards for hydrocarbon analyses.

Although the first committee was formed in 1904, most of the work of the ASTM for petroleum products has been done since 1940. The D-2 technical committee on petroleum products and lubricants is responsible for almost all petroleum products. The D-16 committee is responsible for aromatic hydrocarbons and related chemicals. The E-19 committee for gas chromatography was established in 1961. More recently, it has been expanded to include all types of chromatographic analyses. As such, this committee works closely with the individual product type committees.

The ASTM methods for gas chromatographic analyses usually describe a generalized procedure. They allow for a choice of instrumentation and columns, but set standards for sample preparation, column resolution, and analytical quantification. They therefore provide the analyst with some flexibility to adjust for specific laboratory, company, or personal preferences. At the same time, they maintain standards of practices that can be used to "referee" analytical results between the supplier and the customer.

The origins of tests published by the ASTM vary significantly. Many are developed within organizations such as the American Petroleum Institute (API), U.S. Bureau of Mines, and the National Institute of Standards and Technology [NIST, formerly the National Bureau of Standards (NBS)]. Regardless of their origin, they represent voluntary consensus standards.

The ASTM has published several manuals on hydrocarbon analysis (15–17). These are compilations of all the ASTM standards relating to hydrocarbons and includes the applicable gas chromatographic techniques. A manual on gas chromatographic methods has also been published (18). Volumes 5.01, 5.02, and 5.03 include the updates for petroleum products and lubricants; aromatic hydrocarbons are covered in Volume 6.03; and Volume 14.01 contains the methods for chromatography (19). For further details on the test procedures discussed in this chapter, the most recent annual book should be consulted.

The petroleum industry, like many other industries, has developed its own terminology. The terms used in this chapter reflect those commonly used in the petroleum industry. They are consistent with those adopted by the Division of Petroleum Chemistry of the American Chemical Society. These terms include the following:

- *Aromatics*—all hydrocarbons containing one or more rings of the benzenoid type
- *Naphthenes*—saturated cyclic hydrocarbons or cycloalkanes
- *Olefins*—all alkenes
- *Paraffins*—all noncyclic saturated hydrocarbons, including both normal and branched alkanes
- *Saturates*—all naphthenes and paraffins

13.2 EXPLORATION AND PRODUCTION

The quantitative perspective involved in the earth's carbon chemistry is interesting. There are two parts to the earth's carbon cycle, as illustrated in Figure 13.1. Cycle I is the organic carbon cycle and involves approximately 3×10^{12} tons of fixed organic matter. It has a half-life of several million years and contains a relatively small fraction of recoverable hydrocarbon. Current estimates of 2.1×10^{11} tons of recoverable oil and gas means that only 0.003% of the fixed carbon is available for exploitation.

Petroleum-derived fossil fuels consist of a wide variety of components ranging from methane to high-molecular-weight multifunctional altered biochemical

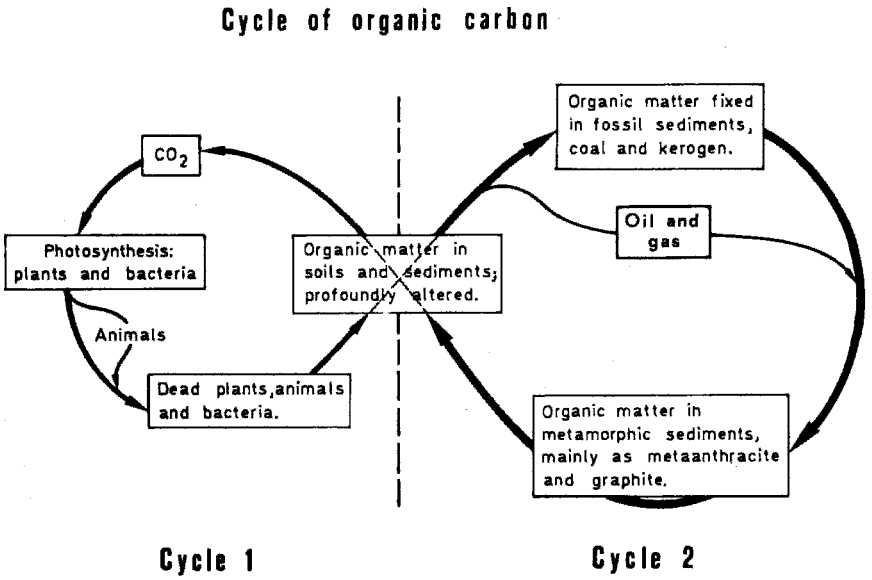

Cycle of organic carbon
CO_2
Photosynthesis: plants and bacteria
Animals
Dead plants, animals and bacteria.
Organic matter in soils and sediments, profoundly altered.
Organic matter fixed in fossil sediments, coal and kerogen.
Oil and gas
Organic matter in metamorphic sediments, mainly as metaanthracite and graphite.
Cycle 1
Cycle 2

FIGURE 13.1 The two major cycles of organic carbon on earth. Most organic carbon is recycled within cycle I. The crossover from cycle I to cycle II is only a tiny leak that amounts to only 0.01–0.1% of the total primary productivity. (Reprinted with permission from Reference 20, Springer-Verlag, Copyright 1978.)

molecules. Some of these molecules contain the heteroatoms oxygen, nitrogen, and sulfur, which are able to complex trace metals such as vanadium, nickel, and iron. The composition, source, and quality of such oils are determined by a complex set of variables. These include the type of organic material originally deposited, the depositional environment, and the time and depth of burial—which, in turn, determines the amount of thermal alteration. Furthermore, the fossil fuels are recoverable only by migrating from their original source to a porous and permeable trap or reservoir structure from which they can be economically produced. The composition of petroleum can be altered further once in the reservoir by a host of physical (e.g., phase separation, asphaltene precipitation), chemical (e.g., thermal cracking, thermochemical sulfate reduction, gas and water washing), and biological (microbial biodegradation) processes.

To better understand the complex composition of petroleum and its analysis, it is useful to briefly survey the processes that convert deposited biological detritus to recoverable fossil fuels (12,13). The fact that petroleum is derived from biological material is clear from its composition. Carbon isotope data and the presence of optical isomers strongly suggest a biogenic origin as does the presence of "biomarkers" or geochemical fossils. For example, there are a whole series of saturated hydrocarbons in petroleum, such as acyclic isoprenoids (e.g., pristane and phytane), the tetracyclic steranes (e.g., cholestane), and the pentacyclic triterpanes (e.g., hopane and oleanane), all of which originate from biomolecules

that are synthesized by bonding together five-carbon isoprene (methylbutane) units. Pristane and phytane are derived from the phytol chain of algal and plant chlorophylls and from bacteriochlorophylls as well as a host of other biological lipids. Cholestane is an altered form of cholesterol, a common membrane lipid in green algae, land plants, and animals. Hopane is the altered form of functionalized pentacyclic lipids that serve the same purpose as cholesterols in bacteria. Other biomarkers have been identified as derivatives of fatty acids, membrane lipids, plant terpenoids, carotenoids (which includes the biological pigments), electron transport biomolecules, and waxes, rubbers, resins, and other biopolymers.

The variables that determine how much organic matter is transferred from the organic carbon cycle to the fixed carbon cycle include the quantity of organic matter originally deposited, the type of this organic matter (terrestrial, marine, etc.), and the depositional environment. The last term includes a number of additional variables such as the rate of deposition, whether the depositional zone was aerobic or anaerobic (with or without oxygen) or euxinic (anoxic with H_2S-rich waters), and the degree and type of biological activity during deposition. Petroleum source rocks are sedimentary rocks with sufficient amounts of preserved organic matter to generate and expel oil and/or gas when heated. The conditions that give rise to source rocks represent a balance between biotic productivity, conditions that promote organic matter preservation (anoxic or euxinic bottom waters, hypersalinity), and dilution by inorganic minerals. Sediments deposited with a high influx of organic carbon may not be sources of petroleum if the water column and bottom sediments are oxygenated, allowing for the oxidation and biological recycling of the carbon.

The conversion of deposited organic matter to petroleum is clearly a complex process. The reactions occur under three thermal regimes: *diagenesis*, *catageneis*, and *metagenesis*. Source rocks that pass through these thermal stages are referred to by geochemists as "immature," "mature," and "overmature" with respect to oil generation. Diagenesis, the first step, occurs at low temperatures ($<80^{\circ}C$) and is the process by which biological molecules are first altered through microbial activity and then through thermal reaction to produce an insoluble organic matrix. This organic matter, termed *kerogen*, is primarily a mixture of crosslinked and modified biological lipids and bioresistive biopolymers that are formed by land plants, green algae, and some bacteria. Part of the kerogen matrix is composed of random polymerization and defunctionalization of small molecules. Kerogen, by definition, is not soluble in common organic solvents and can be isolated by dissolving away the mineral matrix with HCl and HF. Some general characteristics of kerogen are summarized in Table 13.1. Note that the origin of the organic matter from which the kerogen is formed strongly impacts on the composition of the kerogen.

Catagenesis is the thermal degradation of kerogen to produce oil and some natural gas. Weak chemical bonds (C–S and C–O) are the first to break, followed by stronger C–C bonds. Temperatures of $\sim 90-150^{\circ}C$ are needed to optimize oil generation, depending on the type of kerogen present and the duration of burial. During catagenesis, the hydrogen/carbon ratio for the kerogen decreases

TABLE 13.1 Kerogen Types, Occurrence, and Bulk Chemistry

Type	Depositional Setting	Oil vs. gas	H/C	O/C	S/C
I	Lakes, restricted lagoons	Oil	Very high	Low	Low
IS	Lakes with a source of sulfate (rare)	Oil	Very high	Low	High
II	Marine shales	Oil (gas)	High	Moderate to low	Low
IIS	Marine carbonates, evaporites, silicates	Oil (gas)	High	Moderate to low	High
III	Coals, land plant matter transported offshore	Gas	Low	High	Low
IIIC	Oil-prone coals, many are in tertiary source rocks of SE Asia	Oil (gas)	High	Moderate to high	Low
IV	Inert carbon due to oxidation or advance maturity	None	Low	Low to high	Low

as hydrocarbons and NSO compounds are released to the surrounding matrix. This organic matter, which also consists of a very small amount of unaltered hydrocarbons from the originally deposited organic matter, can be extracted with solvents and is termed *bitumen*. As catagenesis progresses, the amount of bitumen increases as hydrocarbons are generated from the thermal degradation of kerogen. In general, hydrocarbons generated early in catagenesis are of higher molecular weight than those generated later in the process, but the actual distribution is highly dependent on the nature of the kerogen.

The point where kerogen has exhausted its potential for liquids generation and where hydrocarbons begin to crack is the beginning of *metagenesis*. Under these conditions, methane is the predominate hydrocarbon generated from kerogen. Methane and wet gas are the predominant hydrocarbons generated from the cracking of petroleum. Metagenesis is counterproductive toward the formation of oil but is the source of much of the natural gas produced.

The timescale for these processes is on the order of millions of years; however, another important process is also occurring. During and after the various states of oil generation, hydrocarbons are constantly migrating. Although the mechanisms of oil migration still require better understanding, two types of migration have been defined. *Primary migration*, also termed *expulsion*, refers to the movement of dispersed hydrocarbons out of the sedimentary matrix in which they originated, that is, the source rock. *Secondary migration* refers to the movement of still highly dispersed hydrocarbons through rock layers other than source rock. These "other" rocks generally have a higher porosity than do those found in the source rock or have a network of microfractures and cracks. If this secondary migration is impeded by an impermeable layer of geologic structure, the petroleum or natural gas will accumulate in a reservoir. In the absence of a

trapping mechanism, migration continues both horizontally and vertically upward toward the earth's surface. If petroleum migrates close enough to the surface to be subject to microbial attack, it may become biodegraded and reenters the surface carbon cycle. Because of the enhanced concentration of hydrocarbons in reservoirs, these reservoirs are the object of investigation for petroleum and structural geologists. Petroleum geochemists aid in the discovery of these reserves by providing an understanding of the potential source rocks, the timing of generation and expulsion, migration pathways, and predictions of oil quality.

13.2.1 Geochemical Studies

Since the early 1980s, traditional petroleum geochemical studies have focused primarily on identifying potential source rocks and correlating these sources to oils and gases within a frontier basin. Analytical techniques have been developed to characterize diagnostic molecular (e.g., biomarkers) and isotopic signatures that allows geochemists to reliably conduct correlation studies. Knowledge of the distribution of a source formation and the petroleum it generated is a key element in defining the petroleum system and in developing targets for frontier exploration.

In searching for the source rock, the geochemist is faced with a unique sampling problem. In many cases, the source formation(s) are buried deep in the subsurface. Source rocks may outcrop in isolated places within the basin, but whether these samples are representative of the more deeply buried rock facies is always a concern. Drill holes offer an opportunity to examine these deeper strata, but from a limited number of locations. However many wells target shallow reservoirs and not deeper source rocks. The ideal downhole samples are cores, but these are expensive to obtain and require special drilling equipment and procedures. They do, however, provide an intact sample of sedimentary layers with easily identifiable strata and accurately determined depth. Most of the samples available to the petroleum geochemist are rock chips known as *drill cuttings*. These cuttings are brought to the surface suspended in the drilling fluid and are separated from the fluid by wet sieving in a shale shaker. Samples are periodically scooped at intervals that are correlated to the drilling depth. These are frequently contaminated with cave-ins and particles that are recirculated within the well before reaching the surface. Drilling fluid additives are also potential sources of contamination. Clearly, a thorough understanding of such a nonideal sample source is necessary for the reasonable interpretation of the analytical results.

The kerogen in cores, cuttings, and outcrop rocks may be examined by a variety of optical and chemical methods to determine its type, thermal maturity, and present-day and original petroleum generative potential. For example, gas chromatographic analyses are used to characterize hydrocarbons that may be cleaved from the kerogen either by thermal energy (pyrolysis) or by selective chemical degradation of specific C–S and C–O bonds. Pyrolysis may be conducted in open or closed systems. In most open systems the kerogen or source rocks are rapidly heated (up to $+600^{\circ}C$) and the pyrolyzates are swept into the chromatograph

for immediate analysis. Closed-system pyrolysis involves sealing the kerogen or source rock in a vessel, either under anhydrous or hydrous conditions, heated for days or hours at temperatures of ~250–500°C, and the evolved hydrocarbons are then analyzed using a modified chromatographic injector. In one variation of closed-system pyrolysis GC, the sealed vessel is a small glass capillary tube that may be directly introduced into the chromatograph (22,23). The pyrolyzates are then introduced into the GC by crushing the tube and sweeping the volatilized matter directly onto the column (Figure 13.2).

Gases and bitumens associated with sedimentary rocks provide a wealth of information concerning the nature of their source. Analysis of light hydrocarbons adsorbed to cuttings or cores is used to screen samples for potential production zones and can provide useful geochemical information. The samples (most often cuttings) are analyzed at the well site or; if analyzed remotely, they must be preserved wet in tightly sealed containers that contain a biocide to prevent bacteriological alteration of the sample. The C4 and lighter hydrocarbons are then sampled either from the container headspace or by one of a combination of thermal, mechanical, or chemical extraction methods. Subsequent gas chromatographic analysis frequently includes backflushing the less volatile C4+ hydrocarbons in order to minimize analysis time while providing the desired analysis of C1–C4 hydrocarbons. Samples with more than 95% methane are called "dry," and are associated with natural gas from either immature (biogenic) or overmature sources, whereas samples relatively high in C2–C4 hydrocarbons (>5%) are referred to as "wet" and are most often associated with the presence of oil.

In an early study of source rocks of western Canada by Evans and Staplin (24), cuttings gas analyses were used to help map areas of immature, mature, and overmature petroleum sources. The progression from immature to early mature shown in Figure 13.3 occurs abruptly at a narrow interval beginning at a depth corresponding to a temperature of 90°F (32°C). The temperatures shown are the paleotemperatures or the maximum temperatures to which the rock was exposed. These are 70–100°F higher than current temperatures because of the uplifting that had occurred.

Another study of the same general area by Bailey et al. (25) offers an interesting comparison of samples representing the progression from immature to overmature sources. Figure 13.4 shows the analysis of C1–C4 hydrocarbons in typical well log form, that is, as a function of well depth. Well 1 shows methane and very little C2–C4 hydrocarbons. This, along with a very light-colored kerogen, indicates an immature source. Wells 2 and 3 show increasing amounts of wet gas and have an intermediate kerogen color, indicating optimum maturity. Well 4, like Well 1, shows primarily methane in the cuttings gas, but a very dark kerogen color indicates that this dry gas is the result of an overmature source. Samples of the same locations were also analyzed for C4–C7 hydrocarbons, that is, the gasoline range (Figure 13.5). These results closely parallel the cuttings gas results.

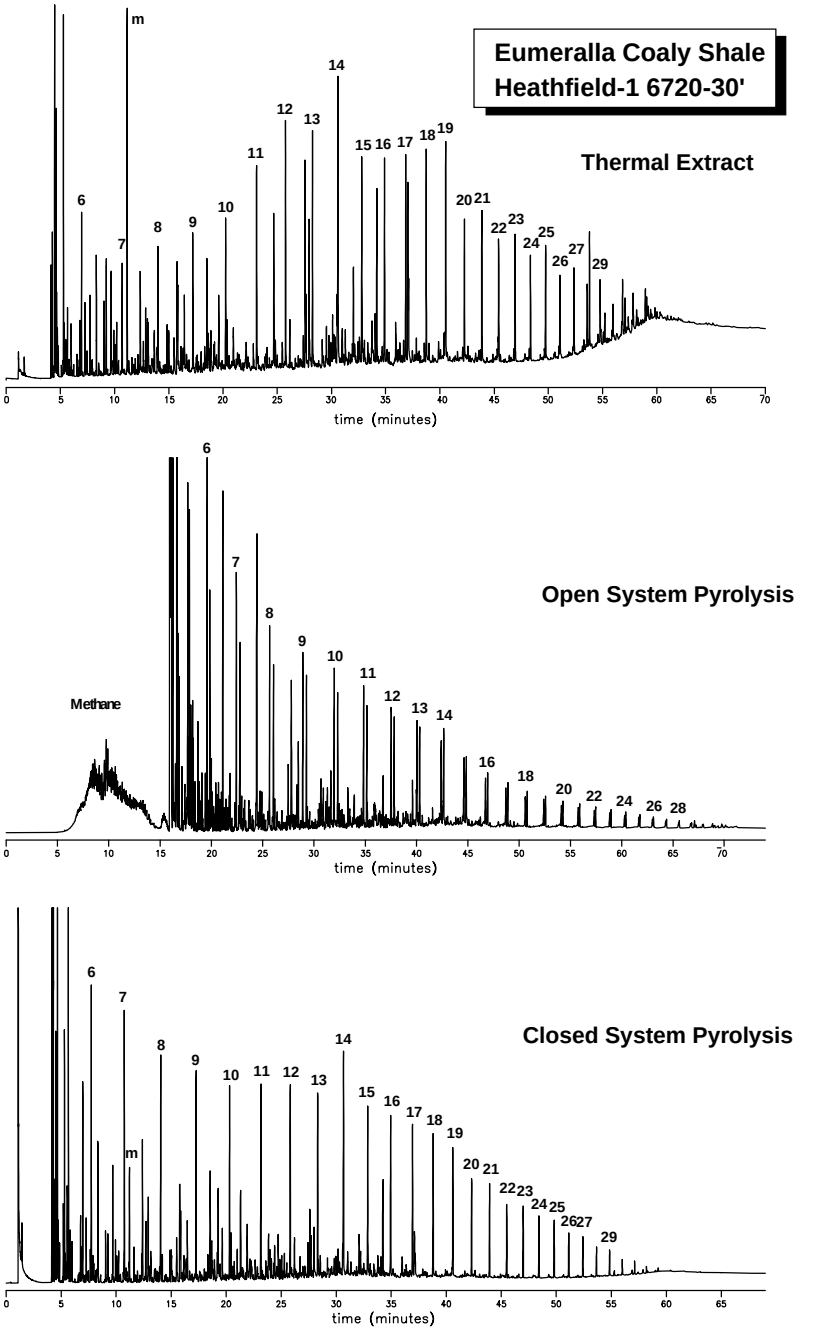


FIGURE 13.2 Thermal extraction, open-system, and closed-system pyrolysis gas chromatograms of the oil-prone coal from the Heathfield-1 Well using the MSSV (microscaled seal vessel pyrolysis) method (see References 12 and 13): m = methylcyclohexane, *n*-alkanes (alkenes) indicate carbon number.

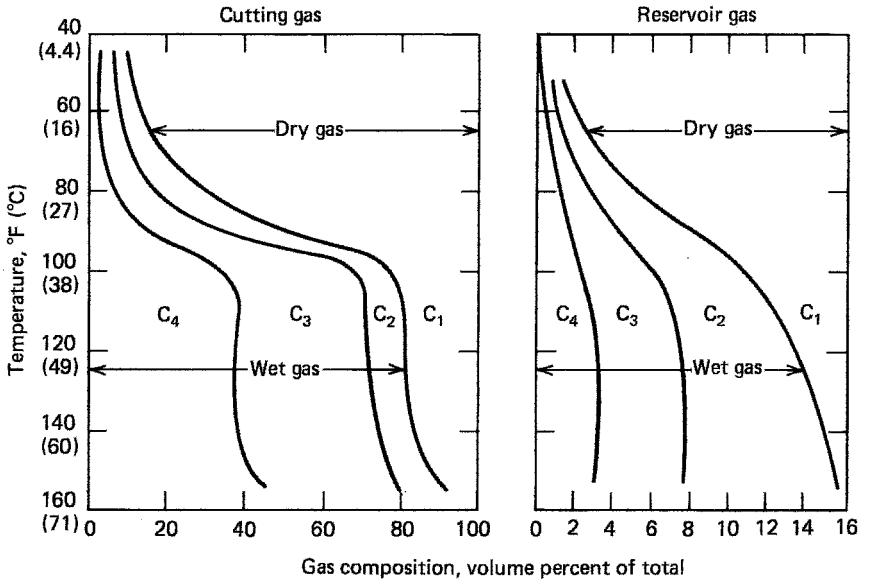


FIGURE 13.3 The composition of Cretaceous gas in source and reservoir rocks versus subsurface temperature. (Reprinted with permission from Reference 24.)

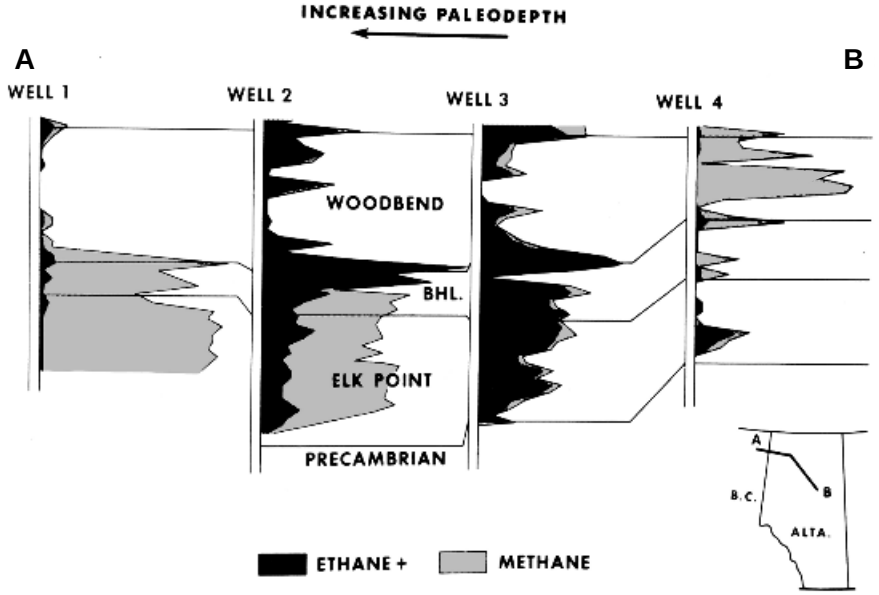


FIGURE 13.4 Cuttings-gas (C1–C4) composition (in log cross-sectional form) of Upper and Middle Devonian strata. The maximum paleodepth increases from east to west. The initial decrease and subsequent decrease in wet gas in this direction illustrate the transition from immaturity to maturity to metamorphism with increasing temperature. (Reprinted with permission from Reference 25.)

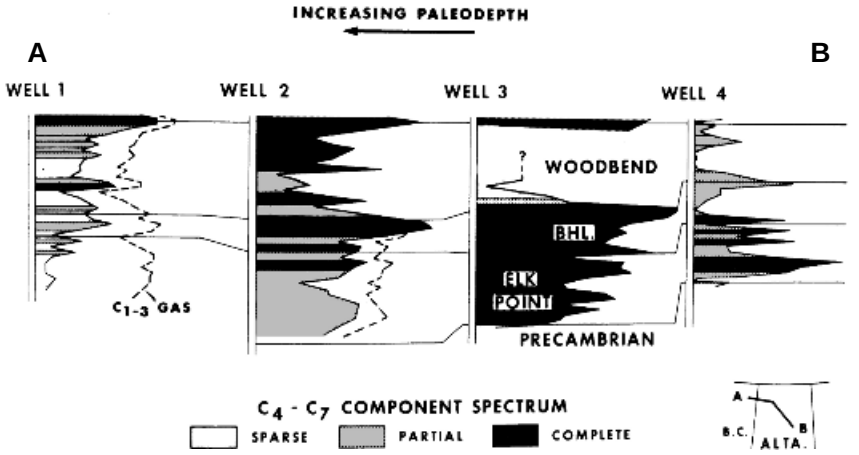


FIGURE 13.5 Gasoline-range hydrocarbon (C4–C7) composition (in log cross-sectional form) for the same wells shown in Figure 13.4. An increase and subsequent decrease both in richness and completeness of the array of components in this fraction illustrates increasing maturation culminating in metamorphism. (Reprinted with permission from Reference 25.)

An early method for the analysis of the C4–C7 hydrocarbons of cuttings or cores involved solvent extraction with a heavy solvent that can be backflushed with the C7+ hydrocarbon fraction. A typical instrument and chromatogram are shown in Figure 13.6. Generally, the choice of solvent is what limits the upper range of the hydrocarbons that are analyzed and so represents a limitation of the method.

The heavy solvent backflush methods have been replaced largely by thermal methods as developed by Schaefer et al. (26,27) whereby they are flushed from the sample by the carrier gas. In this analysis, a small amount (<1 g) of rock chips or powders is placed within a heated injector and the sample is stripped of adsorbed hydrocarbons by the carrier-gas stream (Figure 13.7). The hydrocarbons are concentrated in a cryogenic trap and then vaporized onto a capillary column. A Deans type flow controller (28) may be added, allowing backflushing of the C10+ hydrocarbon fraction while the analytical separation proceeds on the downstream segment of the column. A typical chromatogram is shown in Figure 13.8. In addition to extending the range of hydrocarbons that can be analyzed, this approach reduces sample size requirements and preparation time.

This procedure was used by Thompson (29) to develop C7 source and maturation parameters for a variety of rock and oil samples. Two of these parameters are in common usage: the *heptane index*, which is the ratio of *n*-heptane to the sum of C7 hydrocarbons, and the *isoheptane index*, which is the ratio of C7 isoparaffins to dimethylcyclopentanes. These ratios were found to increase with maturation along source-specific trends.

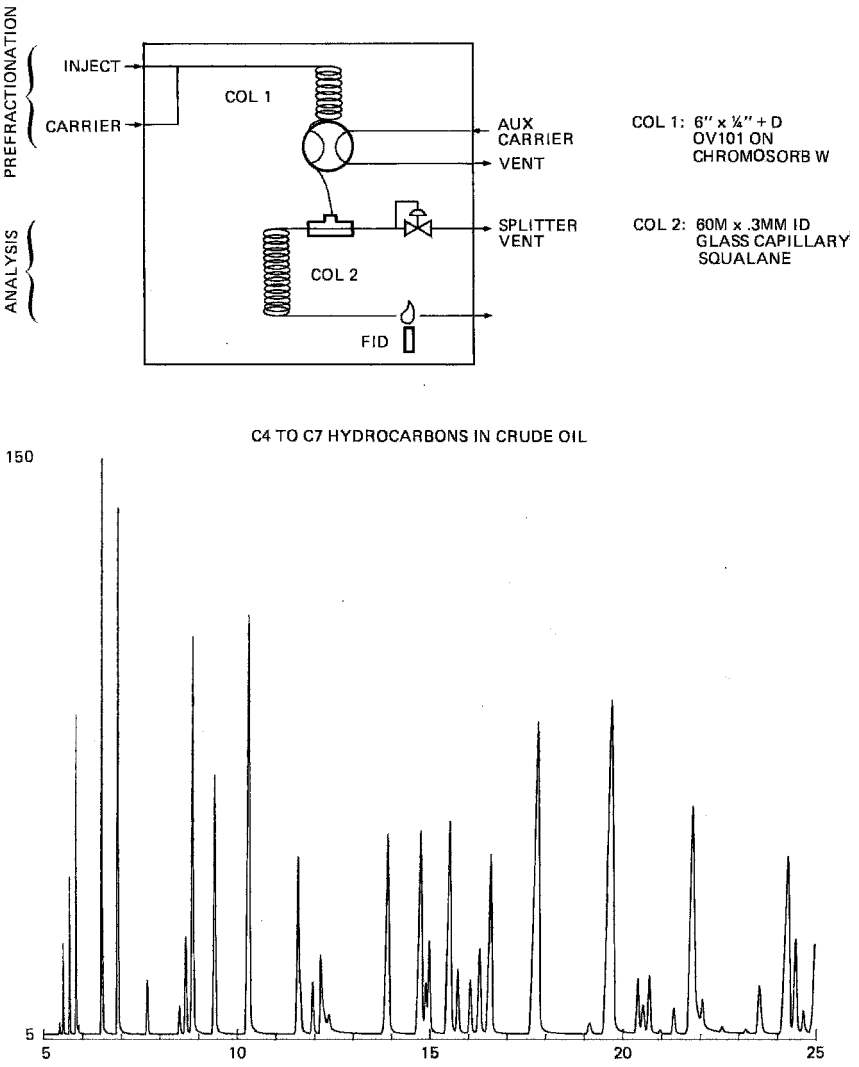


FIGURE 13.6 Chromatographic system and typical chromatogram for the analysis of C4−C7 hydrocarbons in crude oil extracts. The solvent is backflushed with the C8+ fraction.

Light hydrocarbon gases (C1−C4+) leak from subsurface accumulations and may become associated or weakly absorbed in overlaying soils and marine sediments. Surface expressions of anomalous high concentrations of thermogenic gases have been correlated with vertical migration from subsurface accumulations. Geochemical surface surveys can be an effective tool for developing prospects in frontier basins, delineating reservoir boundaries (30), and even monitoring reservoir production (31). Measurement of the stable carbon isotopic ratio

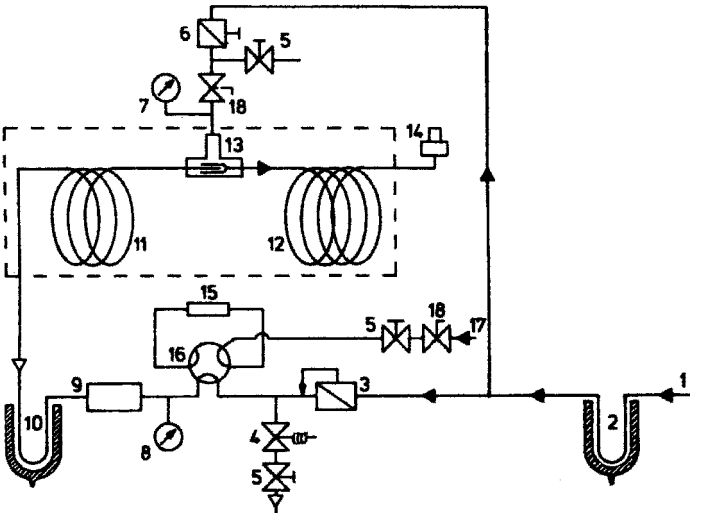


FIGURE 13.7 Modified capillary gas chromatographic system for light hydrocarbon analysis of rock samples by hydrogen stripping: 1, hydrogen inlet; 2, hydrogen purification trap; 3, flow controller; 4, solenoid valve; 5, needle valve; 6, pressure regulator; 7, 8, pressure gauges; 9, sample tube; 10, cold trap; 11, 12, capillary column; 13, T-union; 14, FID; 15, gas loop; 16, six-port valve; 17, inlet for external standard; 18, valve: -►-direction of gas flow, △right-reversed flow during backflush. (Reprinted with permission from Reference 27, *Analytical Chemistry*, Copyright 1978, American Chemical Society.)

(d^{13}C) is used to differentiate biogenic and thermogenic origins of methane in both surface and subsurface samples. Such measurements are now routinely made using gas chromatography–combustion–mass spectrometry hybrid instruments (32).

Oils are the other major source of information for the petroleum geochemist. As the nature of petroleum reflects the organic matter, depositional setting, and thermal maturity of its source, these properties can be inferred reliably in the absence of actual rock samples. Oils may be found as natural seepages on the surface or seafloor or from wells. The latter may be collected during exploratory drilling using downhole sampling tools, or during production. Smaller amounts of free petroleum occur as either stains on reservoir rocks and migration pathways, or as bitumens generated by source rocks. Stains and bitumen (C10+) may be extracted from sedimentary rocks using a variety of solvents and extraction methods. Once extracted, bitumen may be analyzed using the same procedures developed for liquid whole crudes and crude oil fractions.

The basic molecular characterization of oils and extract is chromatographic analysis of the whole sample. This is sometimes called "fingerprinting" and may be a qualitative of semiquantiative analysis. The chromatograms of rock extracts or oils provide the distribution of the major molecular components,

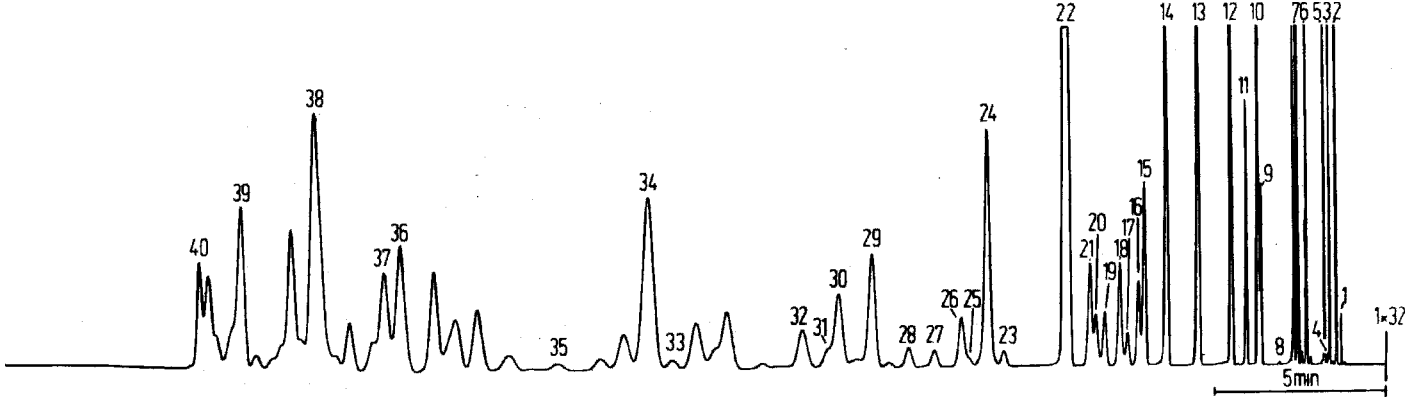


FIGURE 13.8 Typical light hydrocarbon distribution obtained by the hydrogen stripping of a sandy shale core sample (1190 m depth, Lower Cretaceous, northwestern Germany). Peaks: 12, hexane; 14, benzene; 22, heptane; 29, toluene; 34, octane. Complete identification can be found in original reference. (Reprinted with permission from Reference 27, *Analytical Chemistry*, Copyright 1978, American Chemical Society.)

typically light hydrocarbons, normal and branched alkanes, and monocyclic saturated and aromatic hydrocarbons. Routine whole-oil chromatographic methods use 30–60-m fused-silica columns with bonded apolar stationary phases and analyze over a range of ~C4–C40. High temperature GC methods can be used to extend the range to over C80 (33,34). Other geochemical applications of high-temperature chromatographic methods include the direct analysis of geoporphyrins (35), although these compounds are more commonly separated by HLPC methods and detected by MS and MS/MS techniques (36).

Higher resolution of whole oils can be obtained using multidimensional GC or comprehensive two-dimensional GC/GC methods. For example, Walters and Hellyer (37) employed a Siemens SiChromat dual-oven chromatograph equipped with a Deans splitter to develop a the multidimensional method for the fast and complete separation of the C6–C7 light hydrocarbons found in petroleum. The complexity of the composition of petroleum offers an obvious application for the added resolving power of GC/GC techniques (38,39). In GC/GC, oil is separated by volatility using a conventional separation. Instead of being routed directly to a detector (e.g. FID or MS), the effluent is first split into small segments that are then routed to a short second column that has a stationary phase that selects for a different property (e.g., polarity, optical configuration) than the volatility.

The complexity of the minor and trace components, such as adamantanes or biomarker compounds, usually requires that the oil or extract be fractionated into chemical classes and then analyzed by GCMS procedures. Most fractionation methods begin with the asphaltenes separated from the bulk sample by precipitation from pentane or hexane. The deasphated fraction then is separated into saturated hydrocarbon, aromatic and sulfur–aromatic hydrocarbons, and polar NSO (nitrogen-, sulfur-, and oxygen-containing heterocompounds) fractions. This fractionation may be performed via elution from an open alumina–silica column, or now, more commonly, by preparative HPLC systems. The four fractions are then available for subsequent treatment or subfractionation (e.g., urea or zeolite adduction to further separate the normal alkanes from the other saturates). Zeolites also may be used to isolate trace compounds for specific biomarker or isotopic analyses (40). HPLC procedures also are used for the direct separation of oil subfractions such as aromatic hydrocarbons by ring size, sulfides, polar and neutral nitrogen, and acids (41,42).

Once separated, trace petroleum hydrocarbons are further separated by a variety of chromatographic methods. It is here that the analytical approach used by petroleum geochemists and refinery chemical engineers most differs, reflecting specific needs. Geochemists commonly rely on subtle variations in isomer distributions and isotopic ratios of individual compounds to reveal the nature of their source, thermal maturity, and extent of secondary alteration. Refinery chemists emphasize analytical techniques that can be used to group compounds according to their behavior during refinery processes. One of the major differences in analytical approaches is the standardized procedure. In petroleum geochemistry, there is little uniformity in laboratory methods between laboratories. In the quest for better resolution and sensitivity, the latest advances in chromatographic and

spectrometric technologies tend to be quickly adapted without too much regard for compatibility with older techniques.

Gas chromatography, using bonded-phase, fused-silica capillary columns, is the most commonly employed procedure for the separation of minor and trace hydrocarbon components. A variety of detectors are used; the most common is flame ionization for generic quantitation, magnetic sector, and quadrupole MS and MS/MS for biomarker compounds (43), element-specific detectors for heteroatomic and metallocompounds (44), and high-resolution MS for measurement of carbon (45) and hydrogen (46) isotopic ratios. Ultra-high-resolution *Fourier transform* MS (FTMS) coupled with soft-ionization techniques offers the opportunity to detect trace petroleum components without prior enrichment (47).

An early example of the application of GC to petroleum geochemistry is provided by Wehner and Teschner (48). Techniques of GC, gas chromatography–mass spectrometry (GCMS), and high-performance liquid chromatography (HPLC) were used to establish oil–oil and oil–source correlations in the Molasse Basin of southern Germany. Typically the saturate hydrocarbon fraction is chromatographed on a 30-m SP-2100 capillary column with temperature programming from 100 to 270°C. The capillary column analysis of the saturate fraction showed evidence of biodegradation (Figure 13.9). Thus a conclusive determination could not be made from comparison of the biodegraded sample with the others. Liquid

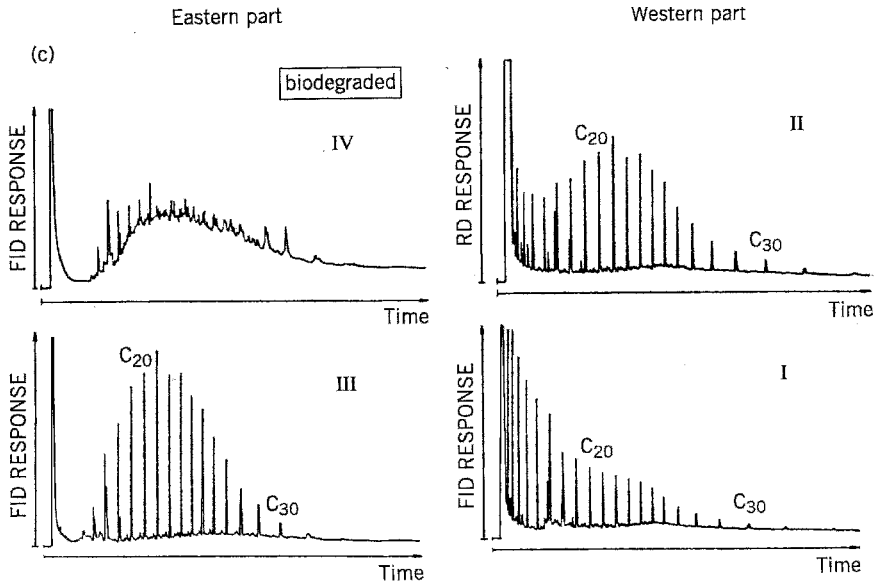


FIGURE 13.9 Gas chromatographic analysis of the saturate hydrocarbon fraction of crude oils from regions in the southern Germany Molosse Basin. Oils labeled II and III have similar patterns of *n*-alkanes. Oil from area IV shows almost no *n*-alkanes, characteristic of biodegradation. (Reprinted with permission from Reference 48, *Journal Chromatography*, Copyright 1981.)

chromatographic analysis of the aromatic fractions and GCMS analysis of the sterane and hopane biomarkers in the saturate fractions provided the additional data needed to complete the correlations.

One of the first studies of the effects of biodegradation on hydrocarbons was made by Jobson et al. (49). Bacteriological degradation proceeded rapidly under controlled conditions. After 21 days, essentially all of the *n*-alkanes were metabolized, leaving behind unaltered iso- and cyclic alkanes and aromatics in the extractable fraction (Figure 13.10).

A classic study by Albrecht et al. (50) used capillary columns (45-m Apiezon L or SE-30) to examine the diagenetic effects on source rocks in Cameroon. Oil generation occurred in a narrow band between 1500 and 2500 m as indicated by the total organic content of sediments in this interval. Gas chromatographic analyses of the C15+ hydrocarbon fraction (Figure 13.11) show a parallel increase in the yield of hydrocarbon as well as a shift in the saturate type and *n*-alkane molecular weight distribution. Saturates at shallower depths are mostly iso- and cyclic alkanes (represented by the unresolved background), indicative of immature sources, whereas the normal alkanes are the predominant type of alkane at more mature intervals. The carbon number distribution also shifts to higher values for the more mature sources. Analyses of the aromatic fractions of these same samples also show a shift in molecular weight with increasing maturity.

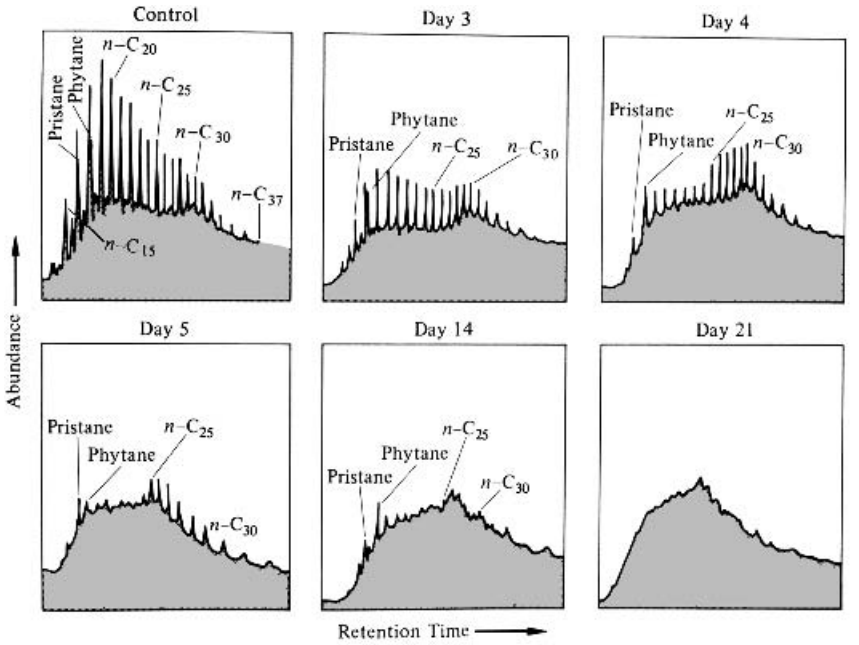


FIGURE 13.10 Gas chromatographic analysis of whole oil shows the disappearance of *n*-alkane peaks, first in the C15–C25 range and later in the entire range during incubation with a mixed microbe population at 30°C. (Reprinted with permission from Reference 49.)

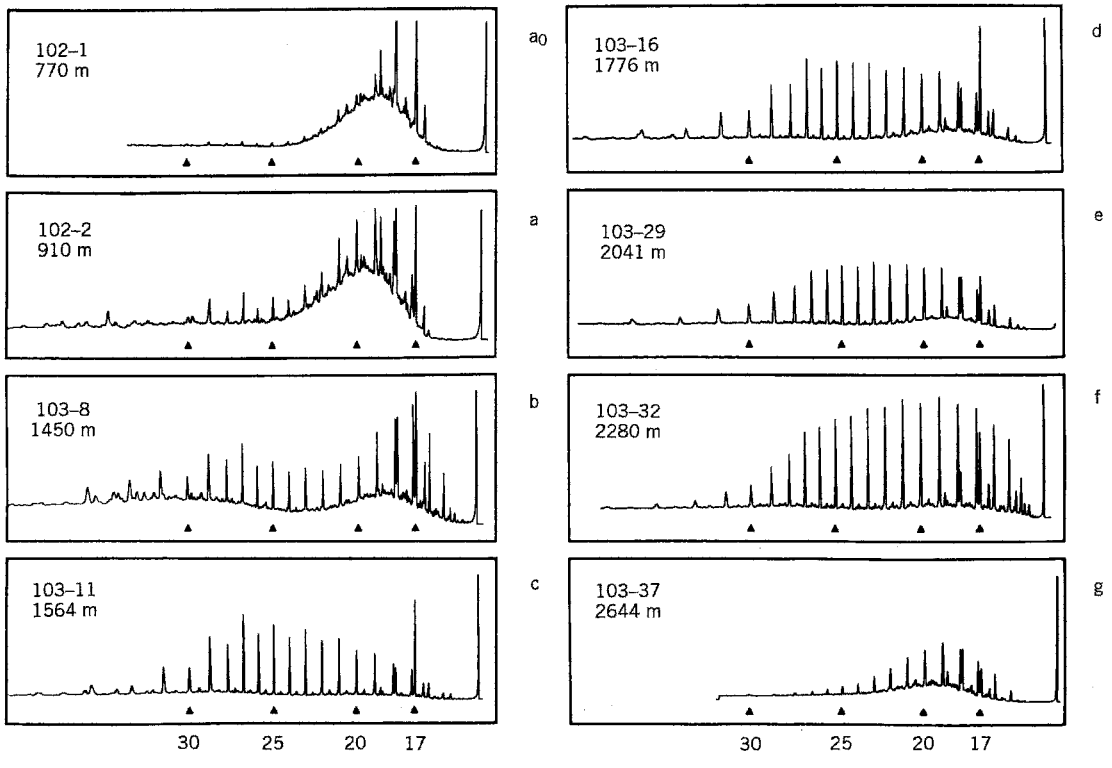


FIGURE 13.11 Analyses of the saturate fractions of oils from various depths showing how composition changes with maturity. Triangles mark from right to left C17, C20, C25, and C30, respectively. (Reprinted with permission from Reference 50, Copyright 1976, Pergamon Press, Ltd.)

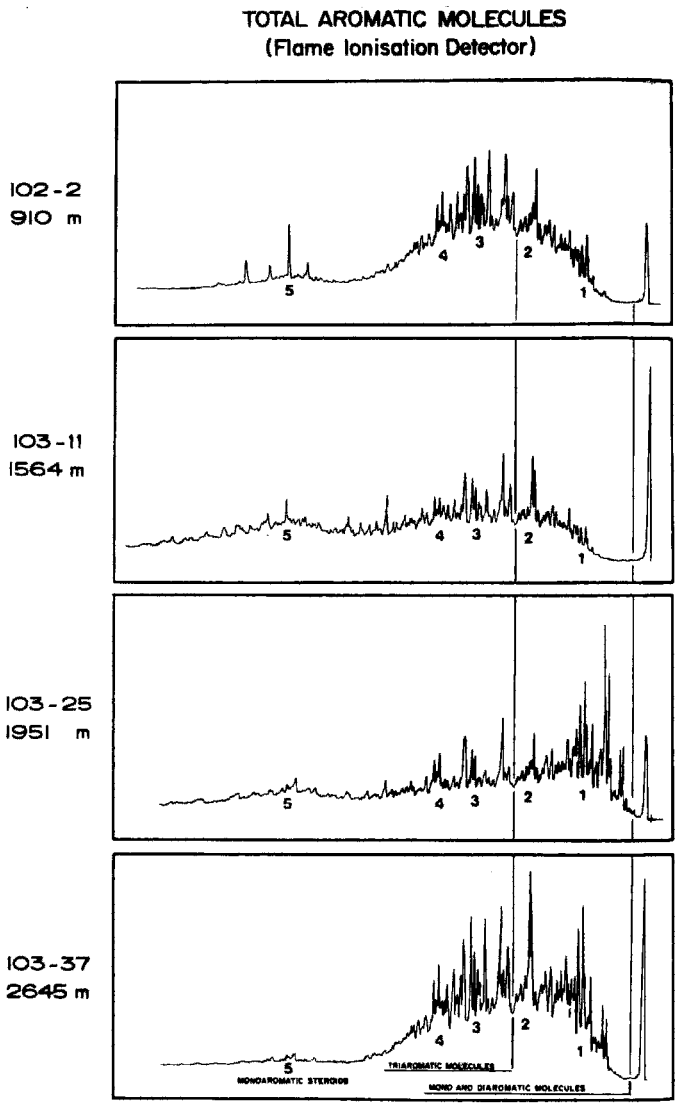


FIGURE 13.12 Analyses of the aromatic fraction of some of the same oil shown in Figure 13.11 showing the change in composition and distribution as a function of maturity. (Reprinted with permission from Reference 50, Copyright 1976, Pergamon Press, Ltd.)

Figure 13.12 shows a decrease in the five- and four-ring aromatic compounds as a function of depth. Artificial maturing of samples from shallower intervals by heat treating them in the laboratory produced hydrocarbons that showed the same trends as the samples taken from greater depths.

With the availability of GCMS and computerized data-processing systems, the routine analysis of biomarkers, such as steranes and terpanes, became possible.

Seifert and Moldowan (51) published an early application illustrating the potential use of these compounds for fingerprinting crude oils. Prior to this, most of the attention in geochemistry was directed toward the major components of saturated fractions, particularly the *n*-alkanes below C40. Subsequent studies have demonstrated that biomarker compounds are very effective not only for oil–oil and oil–source rock correlation but also to determine depositional settings, thermal maturity, degree of alteration, and geologic age (43,52). For example, oleanane, a C30 triterpane, is derived from biochemical precursors that are produced by angiosperm (flowering) land plants. As these land plants became a significant portion of the biomass only since the Late Cretaceous–Early Tertiary, the presence of oleanane is indicative of this younger geological age. Biomarker analysis via GCMS is now routine, relying on common benchtop equipment (53). Another level of specificity can be gained using MS/MS detectors that are capable of separating coeluting biomarker isomers that differ in parent mass (54).

Since the early 1970s, a large portion of the knowledge gained in organic geochemistry can be tied to advances in gas chromatography and its coupling to specific detectors. The technique has proved adaptable to the characterization of a wide range of petroleum compounds. Future developments in column phases, instrumentation, detectors, and data processing/display will undoubtedly be embraced readily by the geochemical community.

13.2.2 Synthetic Crude Oil

Concern regarding the diminishing crude oil supplies has created a great deal of interest in alternative fuel and petrochemical sources. The more similar to crude petroleum the alternative is, the more easily existing processing equipment can be adapted to handle these new feedstocks. The gap between refinery operations and process compatibility is closing from both sides. Refineries are adding new processes to maximize the useful yields from increasingly heavy and low-grade crude oils. Research on "synthetic" crude oil from shale or coal is concentrating on processes that will produce a product as close to natural crude oil as possible.

Shale oil probably has the greatest potential for becoming an alternative to natural crude oil because of its similarity to natural petroleum. The processes of converting a kerogen-rich shale to an oil source parallels the natural maturation process that occurs during diagenesis. Thermolysis or heatsoaking of immature shales and isolated kerogen has been used by geochemists to characterize petroleum source rocks and to understand the maturation process. Generation of oil from shale represents a massive scaleup of this same process. Clearly, the application of gas chromatographic analyses to such products would be the same as those described in the previous section. Burnham et al. (55) determined that the laboratory thermolysis of shales could be used to accurately predict the yields and distribution of hydrocarbons in shale oil production. Chromatograms like those in Figure 13.13 are more complex than those of crude oils because of the presence of alkenes resulting from the more severe conditions used in producing shale oil.

FID detector response
(a) 0.03 °C/min
CS2
7
B1
C1
A2
B2
11
D4
B3
10
13
I3
I2
A6
I4
15
16
I6
I7
C5
18
19
22
25
28
S3
S6
31

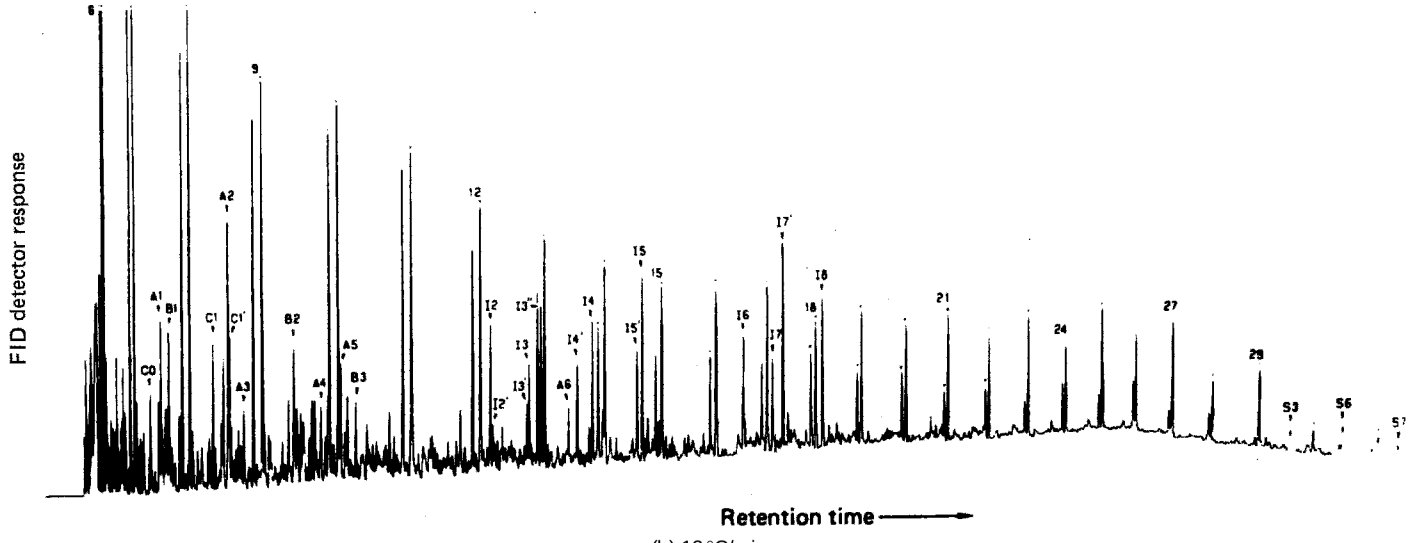


FIGURE 13.13 Chromatograms of shale oil produced at 0.03 and 12°C/min. The shale sample came from Anvil Points, Colorado, and contained 92 mL oil/kg shale. Complete identifications available in the original reference. Normal 1-alkenes and alkanes are indicated by a dot, with the 1-alkenes preceding the corresponding *n*-alkane. (Reprinted with permission from Reference 55, Copyright 1982, Pergamon Press, Ltd.)

Gas chromatography is seldom applied directly to the analysis of tar sands. The bitumen from tar sands represents the heaviest components remaining after the loss of lighter hydrocarbons. Likewise, gas chromatographic analysis is applicable only to the light fractions of coal liquefaction and gasification. Analysis of the gaseous streams of the solvent refined coal (SRCII) process is monitored with an automated gas chromatograph (Figure 13.14). This instrument is a commercially available gas chromatograph that was originally designed for refinery gas analysis. This type of analysis is discussed in more detail in Section 13.3.1.

Lee and his co-workers (57) combined adsorption chromatography and capillary-column GC to characterize the liquid fraction from the SRCII process. A fused-silica column (20 m $\times$ 0.3 mm coated with SE-52) was used along with a flame ionization detector (FID) and either a nitrogen–phosphorous detector or a flame photometric detector. Four hydrocarbon fractions were isolated and characterized. They were found to contain the following functionalities:

- Aliphatic hydrocarbons
- Neutral polycyclic aromatic compounds
- Nitrogen-containing polycyclic aromatic compounds
- Hydroxypolycyclic aromatic compounds

Novotny et al. (58) demonstrated the application of glass capillary columns to the detailed analysis of coal tar samples. An extensive liquid–liquid partition scheme was developed to separate the crude coal-tar sample into basic, acidic, and "neutral" fractions. High-resolution gas chromatography of each fraction yielded a detailed analysis of the original sample. Gas chromatography–mass spectrometry was used to identify fraction components. Identifications were confirmed with authentic compounds where possible. For coal tars, the aromatic fraction represents more than 50% of the original sample. Figure 13.15 shows the chromatogram of this fraction. Figure 13.16 is a chromatogram of the saturate fraction, which, according to the accompanying identification, contains a number of substituted naphthalenes.

13.3 REFINING

Petroleum refining is the process that converts complex crude oils into usable fractions. The process consists of initial separation of the crude into gases, narrow-boiling-range distillates, and bottoms. Some of the fractions are then converted into more desirable components that must be subsequently separated by fractionation. The final refinery products, such as gasoline, kerosene, solvents, lubricating oils, and others, are formed by blending of the various fractions.

A simplified flow diagram of the refinery process is shown in Figure 13.17. The initial crude separation is accomplished by two stages of fractionation. An

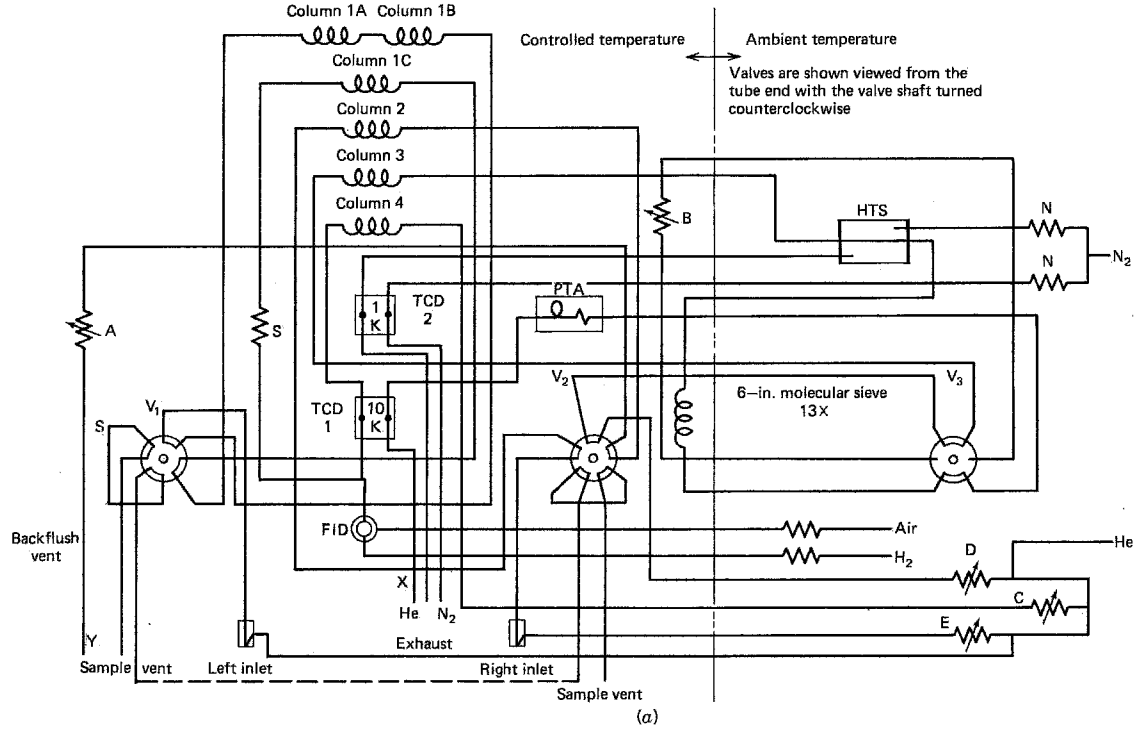
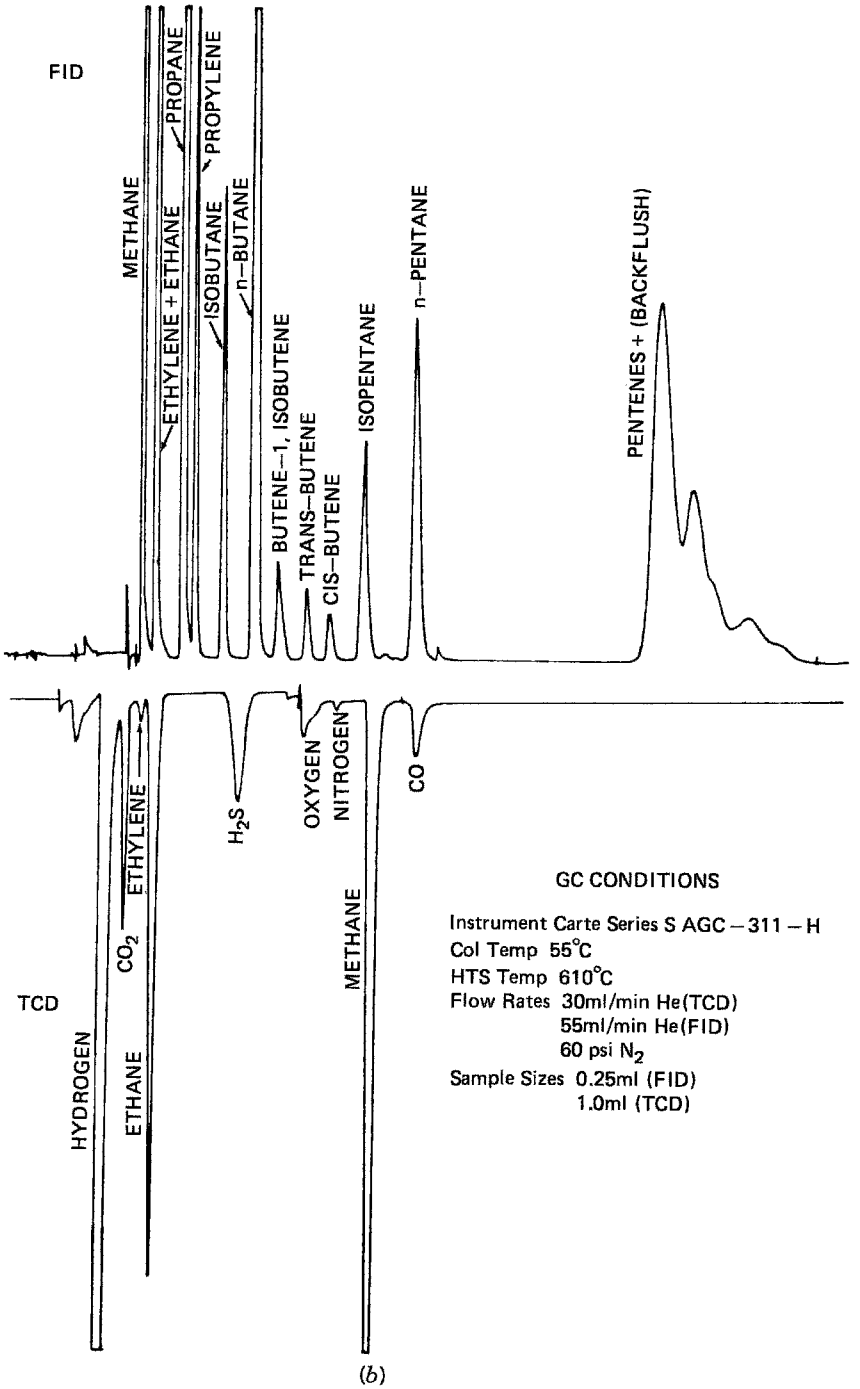


FIGURE 13.14 Typical instrument schematic (a) and chromatogram (b) for the automated gas chromatographic analysis of coal liquefaction gases. Column 1A: 18 ft—27% bis (EE) A + 3.5% Carbowax 1540 + 6.5% DC 200/500 mesh. Column 1C: 12 in. on Chromosorb P-AW 60/80 mesh. Column 1B: 3 ft—OPN/Porasil C 80/100 mesh. Column 2: 6 ft—80% Porapak N + 20% PORAPAK Q 50/50 mesh. Column 3: 7 ft—molecular sieve 13 × 45/60 mesh. Column 4: 6 ft—8% OV-101 on Chromosorb W-AWDMCS, 80/100 mesh. (Reprinted with permission from Reference 56, *Journal of Chromatographic Science*, Copyright 1982, Preston Publications, A Division of Preston Industries, Inc.)



(*b*)

FIGURE 13.14 (*Continued*)

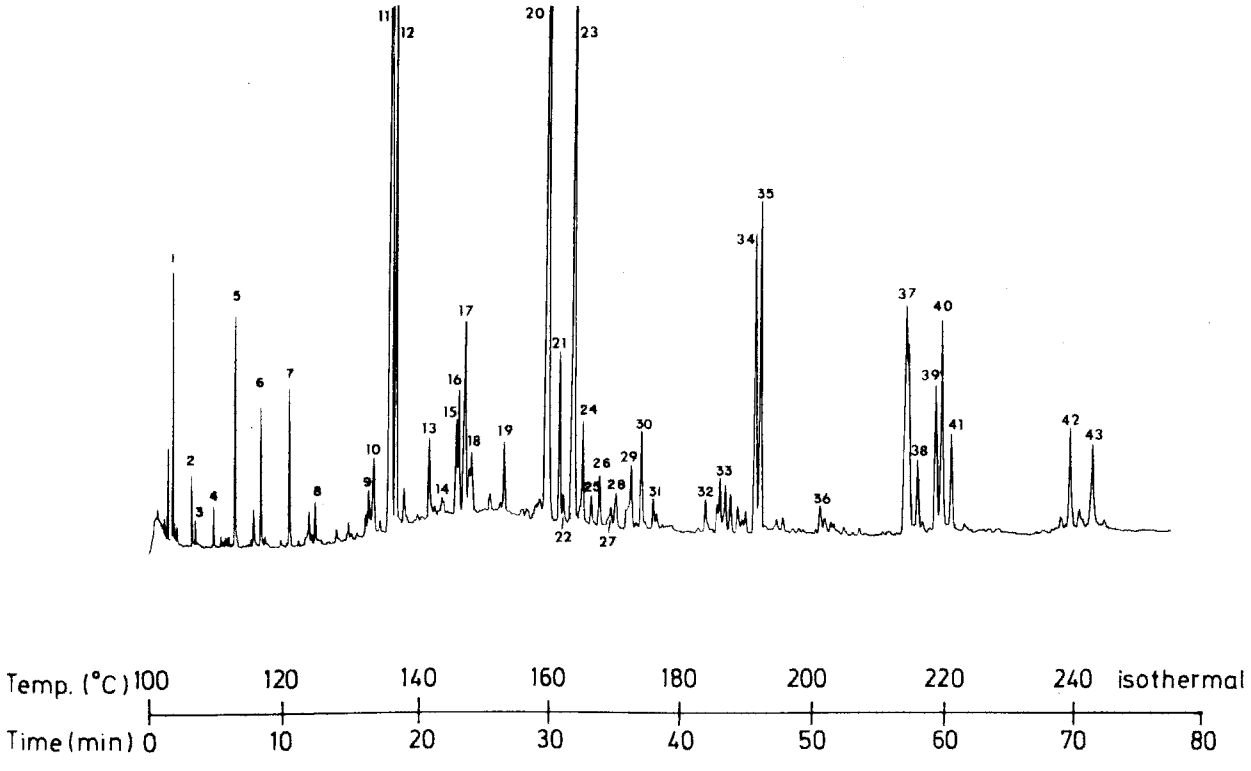


FIGURE 13.15 Chromatogram of the polyaromatic fraction of coal tar. Column 20-m × 0.25-mm glass capillary coated with SE-52. Some peaks identified by mass spectrometry are 1, naphthalene; 12, anthracene; 20, fluoranthrene; 23, pyrene; 41, perylene. Complete identifications are included in the original reference. [Reprinted with permission from Reference 58, *Fuel*, Copyright 1981, Butterworths and Company (Publishers) Ltd.]

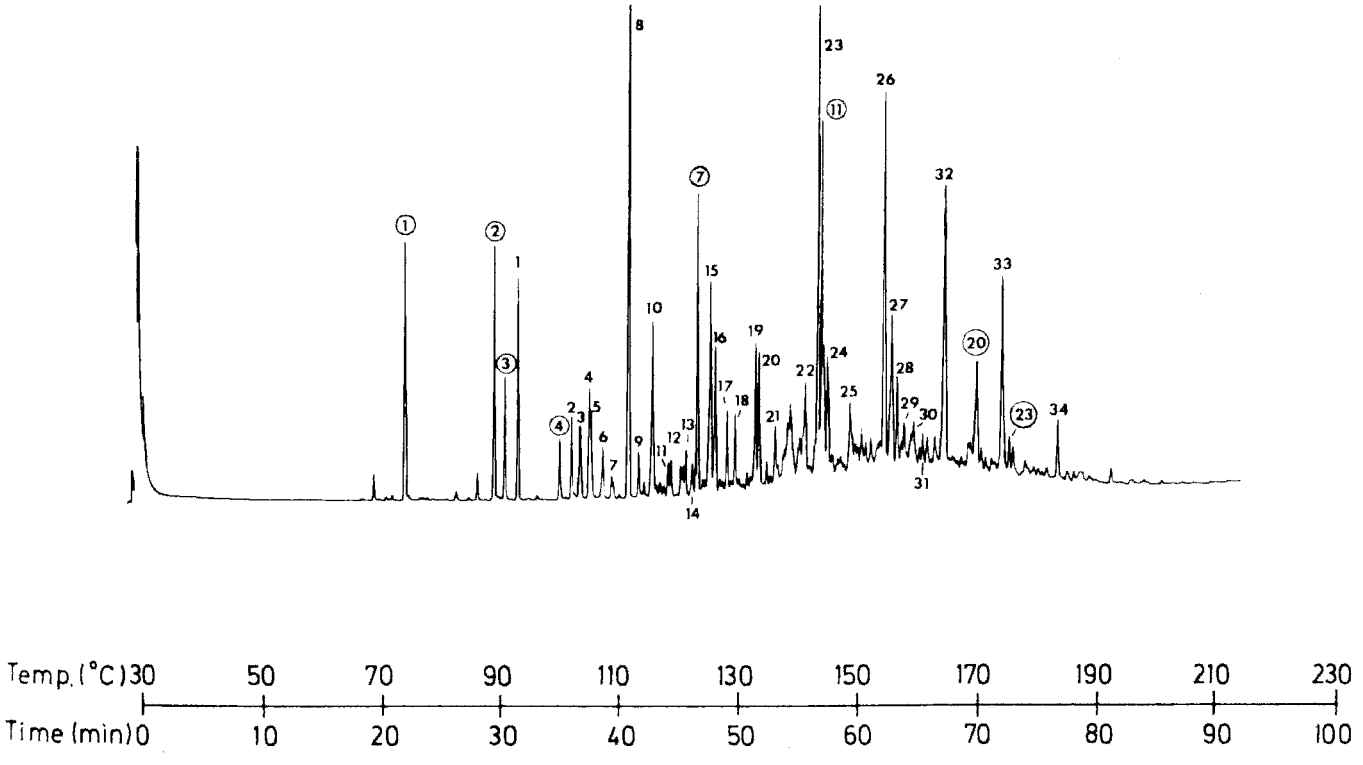


FIGURE 13.16 Chromatogram of the aliphatic fraction of coal tar. Column 20-m × 0.25-mm glass capillary coated with OV-101. Many identified compounds are alkyl-substituted naphthalenes. Peaks: 24, pristane; 27, plythane; 23, *n*-C17; *26*, *n*-C18; *32*, *n*-C19; *34*, *n*-C20. Complete identifications are included in the original reference. (Reprinted with permission from Reference 58, *Fuel*, Copyright 1981, Butterworths and Company (Publishers) Ltd.)

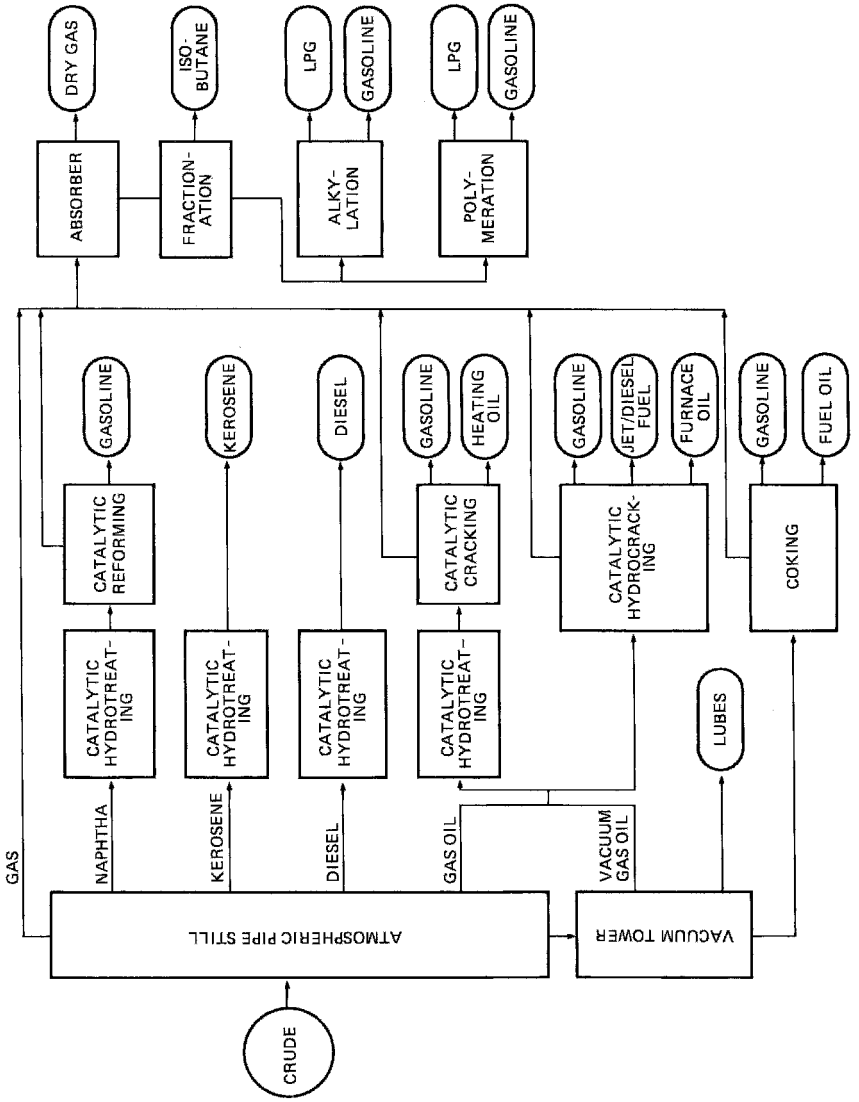


FIGURE 13.17 Simplified flow diagram of a petroleum refining process.

atmospheric pressure tower (commonly referred to as a *pipe still*) separates preheated crude into the following fractions:

Fraction	Boiling Point
Refinery gas–liquefied petroleum gas (C1–C4)	90°F (25°C)
Naphtha–gasoline (C5–C12)	90–400°F (25–204°C)
Kerosene-diesel fuel (C10–C19)	300–525°F (149–274°C)
Light gas oil (C12–C21)	400–650°F (204–343°C)

The bottoms from the pipe still are reheated and further separated in a vacuum tower. Heavy gas oil and lubricating oil cuts are obtained from this tower. The bottoms from the vacuum tower are referred to as *reduced crude* or *residuum* and are used for asphalt and coking. Other common terms used for the distillation fractions include *middle distillates* for light gas oil and *heavy distillates* for heavy gas oil.

Following the initial separation, the various fractions are either sent to blending, separated further, or chemically modified. For gasoline production, which is one of the main purposes of a refinery, the crude fractions are chemically converted to the proper boiling range for gasoline blending. At the same time, this conversion is directed toward the production of higher octane compounds. Octane is a measure of the impact of a compound on engine performance. In general, octane rating follows the progression aromatics>naphthene>isoparaffins>*n*-paraffins. Thus the paraffins in the initial distillation cuts are chemically upgraded through a decomposition process known as *cracking*.

Because of their effects on cracking catalysts as well as the undesirable effects on the final product, sulfur compounds are removed from the distillation cuts before further processing. This is accomplished by catalytic hydrogen treating (hydrotreating). In this step, the sulfur is removed as H_2S. Cracking is basically a process that reduces the size of the molecules and in turn produces a high yield of unsaturates. This is accomplished through high temperatures. High pressures are also required to maintain a totally liquid phase. Catalytic cracking utilizes a solid supported catalyst in a fluidized bed. This accelerates the thermal cracking process by three orders of magnitude. Hydrocracking is a type of catalytic cracking in which hydrogen is added to produce isoparaffins and aromatics. Reforming is a milder operation for lighter fractions that also utilizes a catalyst. It dehydrogenates naphthenes and isomerizes naphthenes and paraffins. Aromatics are the predominate product from this step. Coking is the final type of cracking that thermally decomposes the heaviest fractions. This is accomplished through extensive recycling of the heavy components.

Additional fractionation is required to separate the cracking byproducts into their appropriate boiling ranges for product blending. All the C4 and lighter hydrocarbons are compressed and sent to absorbers. The butanes are removed by absorption into gasoline blend cuts. The lighter components are then fractionated.

Propane and butane are sold mainly as liquefied petroleum gas (LPG). The lightest components are burned as fuel in place of natural gas.

The olefinic hydrocarbon gases, propylene and the butylenes, are sent to polymerization and alkylation. Polymerization forms dimers and trimers of these olefins for use in gasoline blending or for petrochemicals. Alkylation is a more common process in which the olefins are reacted with isobutane. This step produces the more desirable isoparaffins for gasoline blending.

From this brief overview of the refining process, it becomes apparent that the products as well as the process streams for petroleum refining are very complex. Most of these streams are well suited for gas chromatographic analysis. Some of the main applications of GC to these streams and products are discussed in the following sections.

13.3.1 Refinery Gases

Refinery gas analysis involves the determination of permanent gases, hydrogen, all the individual C1−C5 hydrocarbons, and the hexane and heavier content. Streams with varying levels of these components must be analyzed for process control. Natural gas and other streams used for furnace firing must also be analyzed for heat content. Compliance with environmental regulations requires analysis of flue gases and other emission sources. Besides the diversity in composition and origin of samples, sampling is an additional problem. Sampling of multiphase streams over a wide range of temperatures and pressures is often required. Reviews of these sampling and analytical problems have been published by Harvey (59) and Cowper and DeRose (60).

For samples near atmospheric pressure, a glass sample cylinder or rubber bladder may be used. The preferred method, however, is the use of Teflon- or Mylar-coated bags. These bags are easy to handle and are inert to sulfur compounds. Most streams, however, require sampling with stainless-steel cylinders. These are also available with Teflon linings for reactive components. The metal cylinders may be installed on an inline basis with a slip stream of the process stream to ensure representative sampling. Care must be taken to allow for a vapor space in the cylinder when sampling liquids or high-pressure liquefied gases. This prevents overpressuring due to liquid expansion with temperature changes. Safety valves are often installed for this purpose. With proper safety precautions, metal sample cylinders may be heated in an oven to revaporize samples that may condense at ambient conditions.

Analysis of specific components or classes of components in refinery gases can be accomplished with single-column analyses. However, combinations of columns and valving are required for more complete analyses. The various aspects of hydrocarbon gas analysis have been discussed by Thompson (61). Applicable columns for these applications can also be found in column supplier catalogs and the reviews by Mindrup (62) and Leibrand (63).

Analyses for the fixed gases and light hydrocarbons are required for monitoring of stack or flue combustion gases. The concentration of hydrogen in samples is

important for control of cracking and hydrotreating. Oxygen and carbon monoxide must be determined to avoid combustion and other side reactions. A 5A or 13X molecular sieve column with argon or helium as the carrier gas and a thermal conductivity detector is commonly used for the analysis of H_2, O_2, and CO in hydrocarbon streams. When a molecular sieve column is used, the hydrocarbons heavier than methane are normally backflushed from the column. Care must be taken to avoid deactivation of the molecular sieves with water and large amounts of CO_2. Also, isobutane can interfere with the determination of oxygen. Several alternative column packings include Porapak Q, which is also capable of determining water, carbon dioxide, and the other hydrocarbons. Chromosorb 102 and Carbosieve S columns are also suitable.

For determination of the fuel value of refinery gas streams or natural gas, the inert gases must be determined along with the hydrocarbon components. By determining the mole percent concentration of each component, the calorific (heating) value and specific gravity of a gas can be calculated. This information is used to determine the sales value of natural gas. Stufkens and Bogaard (64) used a Porapak R column for the analysis of methane-rich natural gas. A thermal conductivity detector and FID were used in series to determine the nonmethane components. The response of the two detectors was normalized on the basis of the ethane concentration. The ASTM methods for fuel value determinations use two columns. Analysis of natural gas by method D1945 (19) specifies a molecular sieve adsorption column for O_2, N_2, and methane. The C2–C5 hydrocarbons and CO_2 are then determined with a partition column such as BMEE [bis-(2(2-methoxyethoxy)ethyl)ether], silicone 200/500, or diisodecylphthalate dimethylsufolane. For reformed gas containing only C2 and lighter components, method D1946 (11) uses a Porapak Q column for the C2 hydrocarbons.

Because of the harmful effects of sulfur compounds on cracking catalysts, refinery distillation cuts are hydrotreated to convert the sulfur compounds primarily into H_2S. The sulfur content of stack gases must also be monitored for compliance with air-pollution standards. Because of their high polarity and reactivity, inert sampling and column materials must be used to avoid losses and peak distortion. For higher levels, a thermal conductivity detector can be used with a silicagel, Porapak Q, or Carbosieve B column. Levels below 50 ppm require the use of a flame photometric detector (FPD). Pearson and Hines (65) used an FID in series with an FPD for determining trace levels of H_2S, COS, CS_2, and SO_2. They used the FID to verify that the hydrocarbons in the sample were completely separated from the sulfur compounds. This is necessary because hydrocarbons reduce the signal of the FPD. For streams containing C1–C4 hydrocarbons, several columns were used to achieve resolution of the sulfur compounds. These columns included polyphenyl ether–H_3PO_4 on Chromosorb G, silicagel, and QF-1 on Porapak QS.

Complete systems have been developed for the total analysis of refinery and natural gases. These all utilize automatic column switching and multiple detectors. The Universal Oil Products (UOP) method (66) was one of the first of these

systems. It utilized three columns (diethylene glycol adipate plus diethylene glycol sebacate, Porapak Q, and 13X molecular sieve) with valves for backflushing and a single thermal conductivity detector (TCD).

An excellent example of a more recently developed system for refinery gas analyses is available from Wasson–ECE Instrumentation (67). A Hewlett-Packard gas chromatograph equipped with three independent systems that operate simultaneously provides a total analysis in 25 min. One subsystem uses a gas sampling/backflush valve, packed columns, and a FID for the analysis of olefins with an initial C5–C6+ composite backflush. The second subsystem uses gas sampling/switching valves, packed columns, and a TCD for CO_2, ethylene, ethane, acetylene H_2S, O_2, N_2, methane, and CO. The third subsystem uses a gas sampling/switching valve, packed columns, and a second TCD for analysis of hydrogen down to 100 ppm. An extended refinery gas analysis is also available, which utilizes a capillary column in the first subsystem to resolve the C1–C5 paraffins and olefins while reducing the analysis time to 15 min (68). A typical chromatogram is shown in Figure 13.18.

13.3.2 Simulated Distillation

Because refining is primarily a distillation process, laboratory distillations are commonly used to characterize crude oils and process streams. Gas chromatographic data are now being used with increasing frequency to provide the same type but better quality data than those provided by more time-consuming manual distillations. Although not without some disadvantages, simulated distillation continues to gain general acceptance as the method is improved and correlations with manual distillations are developed. ASTM method D2887 was established in 1973 to standardize the use of simulated distillation for distillate fractions. This method was intended to supplement the manual distillation procedures long used in the petroleum refinery: ASTM D86 (Engler distillation), ASTM D1160 (low-pressure version of D86 for heavier products), and ASTM D2892 (a complete "true boiling point" distillation). ASTM method D3710 has also been established for determining the boiling range distribution of gasoline and its fractions by GC (19).

Differences between the simulated and physical distillation procedures lie in the imperfect nature of each. The most frequently used D86 and D1160 methods are relatively fast single-plate distillations that closely approximate the refinery processes. The large scale of the refinery processes are of necessity imprecise. Thus the compromise between analysis time and the need for information of adequate precision for process control was met by the single-plate laboratory distillations. The introduction and use of simulated distillations from chromatographic data paralleled the need for more precise and detailed data to optimize the refinery process. Refinery operations have become more costly with the rise in energy costs and the increased value of refinery products.

Eggerston et al. (69) in 1960 first reported that low-resolution, temperature-programmed, gas chromatographic data could be used to simulate the more time-consuming true-boiling-point distillation. Retention times were correlated

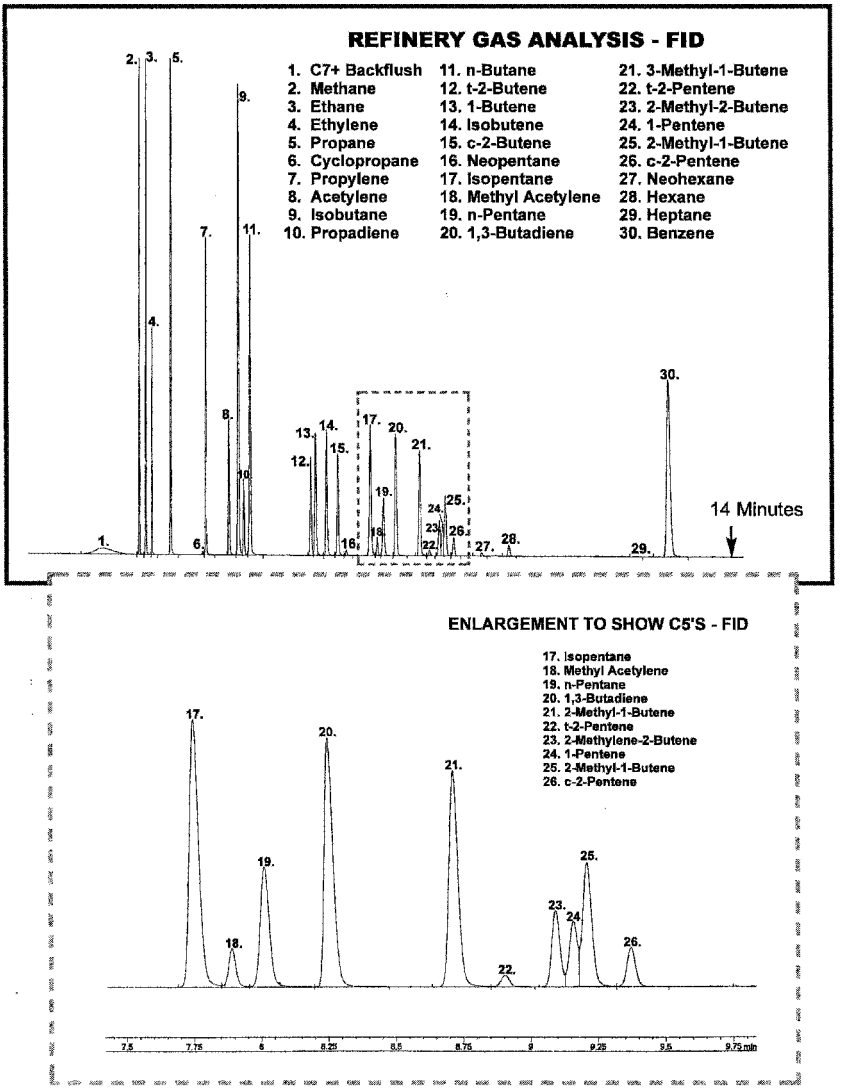


FIGURE 13.18 Typical chromatogram from a standard refinery gas analyzer with simultaneous TCD/FID: (a) refinery gas analysis, FID output; (b) enlargement of C5 fraction of FID output; (c) refinery gas analysis, TCD output. (Reprinted with permission from Reference 68, Wasson–ECE Instrumentation.)

to boiling point and detector response was correlated to the amount of material "distilled." This was confirmed by Green et al. (70), who first used the phrase "simulated distillation by gas chromatography."

Separations by boiling point are typically obtained on columns with silicone gum liquid phases. These liquid phases include OV-101, UC-W98, UCW-982,

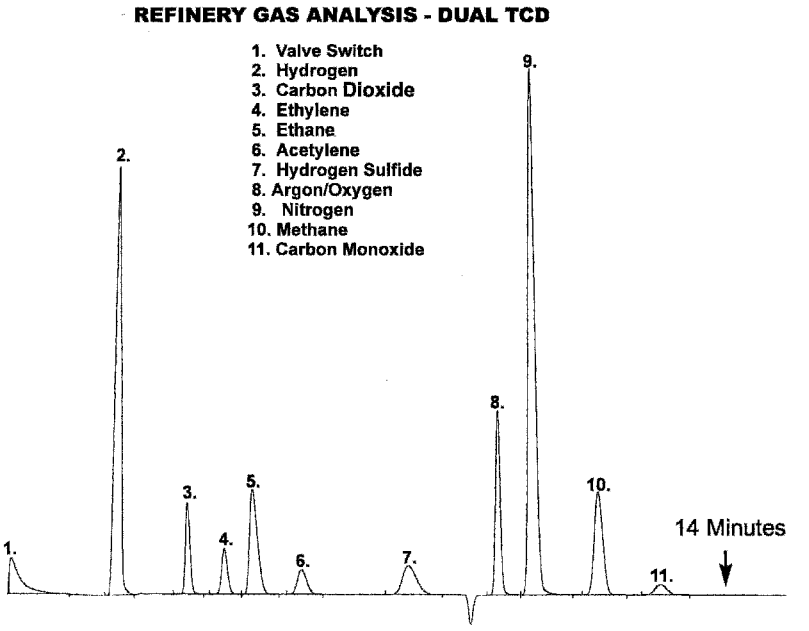


FIGURE 13.18 (*Continued*)

and Supelco 2100 (Petrocol A and B columns). Relatively high liquid-phase loadings (10%) are often used to increase the sample capacity of the column. This minimizes column overloading, but results in a dependence of retention time with the concentration of the sample components. Through the use of temperature programming, a calibration is established on the basis of retention times for a series of *n*-paraffins versus their boiling point, as shown in Figure 13.19. For determining lighter components (C3–C5 hydrocarbons), programming from an initial temperature of $-30^{\circ}C$ is required. The calibration blend can also be used to establish relative response factors. Column resolution must then be monitored along with baseline drift. To compensate for column bleed, a blank run is determined, stored, and then subtracted from subsequent sample runs. Aromatic compounds are used to verify the low polarity of the column.

Sample analyses do not require complete resolution of individual components. In fact, the column length and packing are chosen to obtain a limited resolution that will give a good comparison with the boiling point distribution. A minimum first peak retention time is required to allow the SIMDIS software to establish a proper baseline to ensure an accurate initial boiling point (IBP). An inert packing is used to elute components according to their boiling point and to avoid skewing. Finally, a stable baseline is required to determine when the sample has completely eluted and to determine the final boiling point (FBP).

A typical chromatogram for gasoline by ASTM method D3710 is shown in Figure 13.20. The boiling point distribution is determined by integration of the

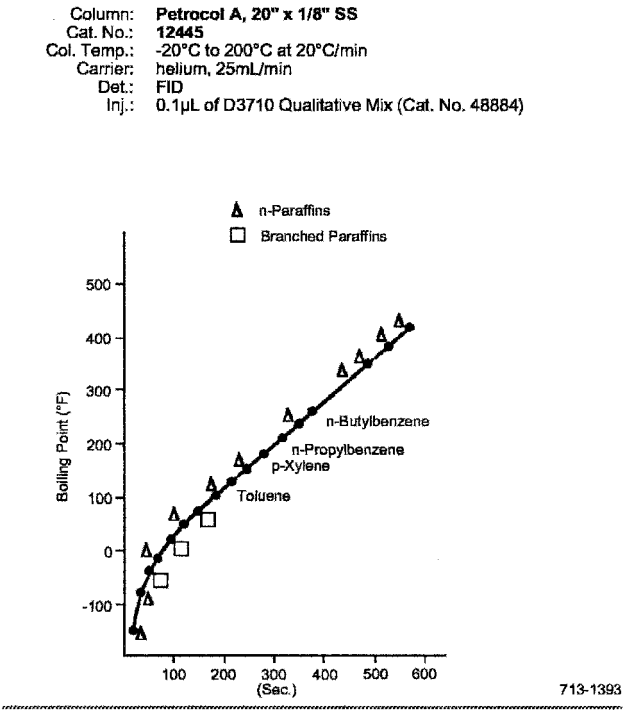


FIGURE 13.19 Simulated distillation calibration curve using a Petrocol A column, 20-ft $\times \frac{1}{8}$-in. SS column. Temperature: -20 to 200°C at 20°C/min. (Reprinted with permission from Reference 71, Supelco, Bellefonte, PA 16823.)

chromatogram by sequential time slices. A distillation curve can then be constructed from the calculated percent off versus boiling-point data. Correlations can also be made with ASTM method D86 (manual distillation) and Reid vapor pressure. The volume percent concentrations of the C3–C5 hydrocarbons are calculated to determine the vapor pressure of the gasoline sample. Many of the chromatographic data systems are capable of handling these calculations automatically.

For heavy distillates, the boiling point distribution is determined by ASTM D2887, as shown in Figure 13.21. This analysis is based on a C5–C44 hydrocarbon calibration. A low-liquid-phase loading (3% versus 10%) is required to allow for elution of samples with final boiling points as high as 1000°F. With this high-temperature range, a stable baseline is critical. A correlation to ASTM D1160 (manual vacuum distillation) rather than ASTM D86 is used for this sample because of its high boiling range.

The ASTM methods have been updated to include capillary-column technology. ASTM D3710 is similar to D2887 as both use external standards composed

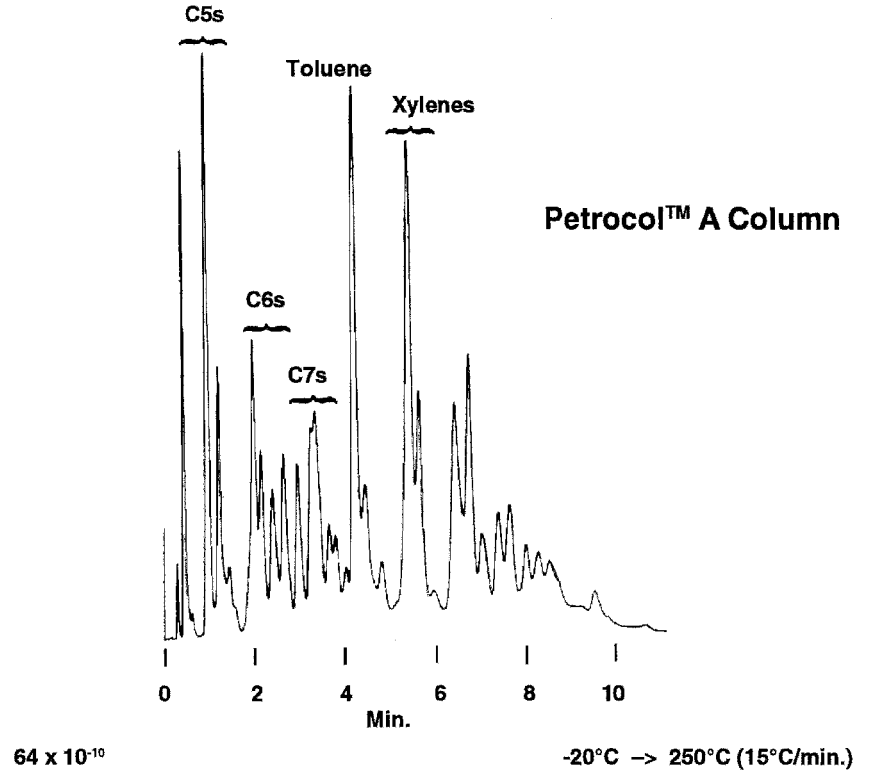


FIGURE 13.20 Simulated distillation chromatogram of a commercial gasoline according to ASTM D3710 using a 20-in. × $\frac{1}{8}$-in. Petrocol A column −20 to 250°C at 15°C/min. (Reprinted with permission from Reference 71, Supelco, Inc, Bellefonte, PA 16823.)

of *n*-alkanes. ASTM method D5307 resembles D2887, but requires two runs for each sample, one of which uses an internal standard. The amount of material boiling above 560°C (reported as residue) is calculated from the differences between the two runs. A SIMDIS chromatogram of a gasoline sample is shown in Figure 13.22. Capillary columns with bonded stationary phases offer advantages including inertness and a more stable baseline due to low column bleed. Longer column life is obtained because large-volume sample injections cannot wash the stationary phase away. Longer retention of early-eluting hydrocarbons give a more linear boiling point/retention time curve. However, this higher resolution presents some problems with low-resolution correlations. Column conditions and SIMDIS software can be used to minimize differences. Otherwise, the higher resolution can be used to provide compositional information on the sample.

High-temperature gas chromatographic (HTGC) analyses are capable of equivalent boiling points in excess of 750°C and have been approved in ASTM D6352. In order to avoid column bleed, the DB-HT SIMDIS column has a thin film that is polymer-bonded and crosslinked with dimethylsiloxane (72). Also, the high

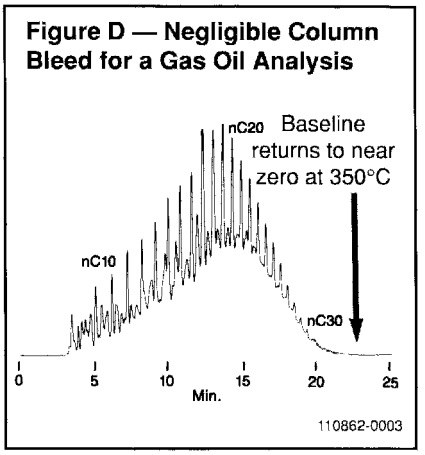


FIGURE 13.21 Simulated distillation chromatogram of a reference gas oil sample according to ASTM D2887 using a Petrocol B column (20-in. × $\frac{1}{8}$-in. SS column with 3% SP2100 (methylsilicone) on 80/100 Supelcoport). (Reprinted with permission from Reference 71, Supelco, Inc, Bellefonte, PA 16823.)

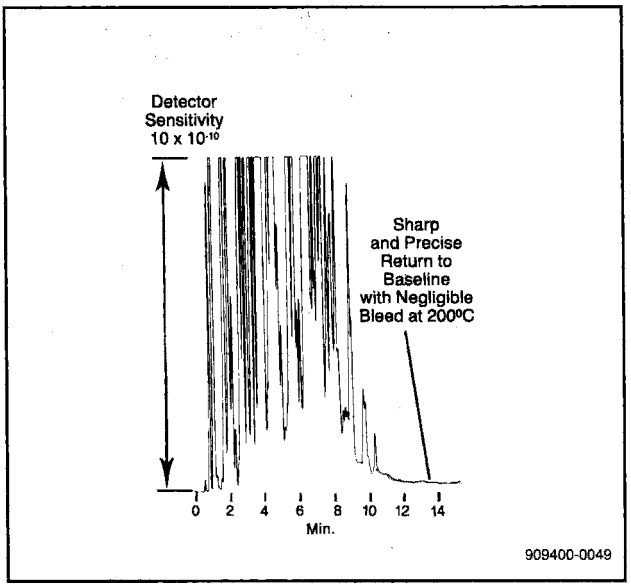
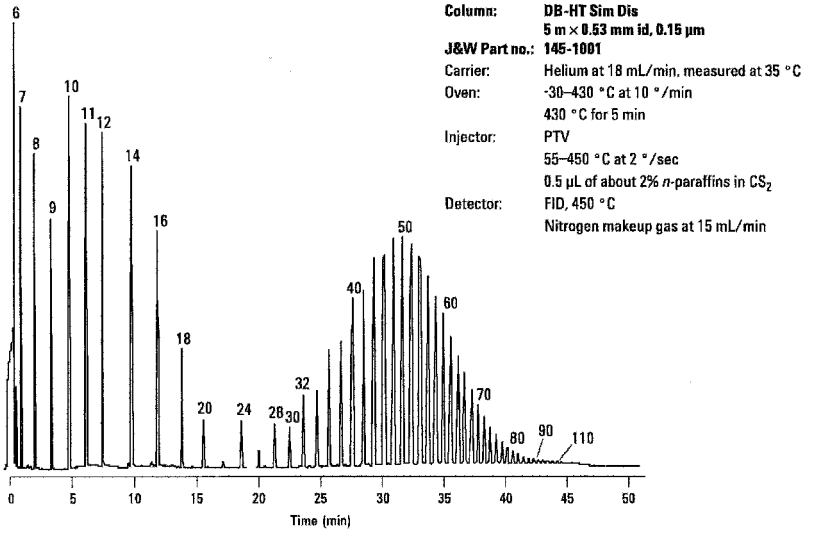
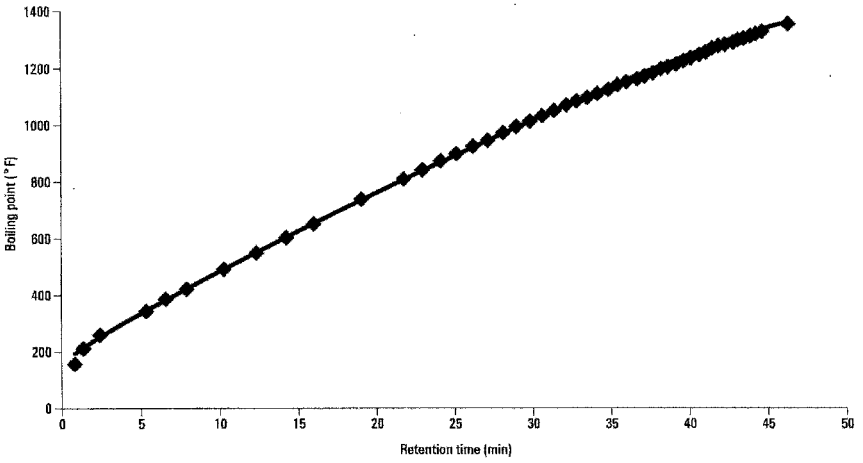

Detector Sensitivity 10 x 10-10
Sharp and Precise Return to Baseline with Negligible Bleed at 200°C
0
2
4
6
8
10
12
14
Min.
909400-0049
Petrocol 3710, 10m x 0.75mm ID glass, 5.0µm film, Col. Temp.: -20°C to 200°C at 20°C/min., Flow Rate: 15ml/min., N2, Sample: 0.1µl of a commercial gasoline.

FIGURE 13.22 Simulated distillation chromatogram of a commercial gasoline using a Petrocol 3710 capillary column (10-m × 0.75-mm-i.d. glass, 5.0-µm film). (Reprinted with permission from Reference 71, Supelco, Inc, Bellefonte, PA 16823.)



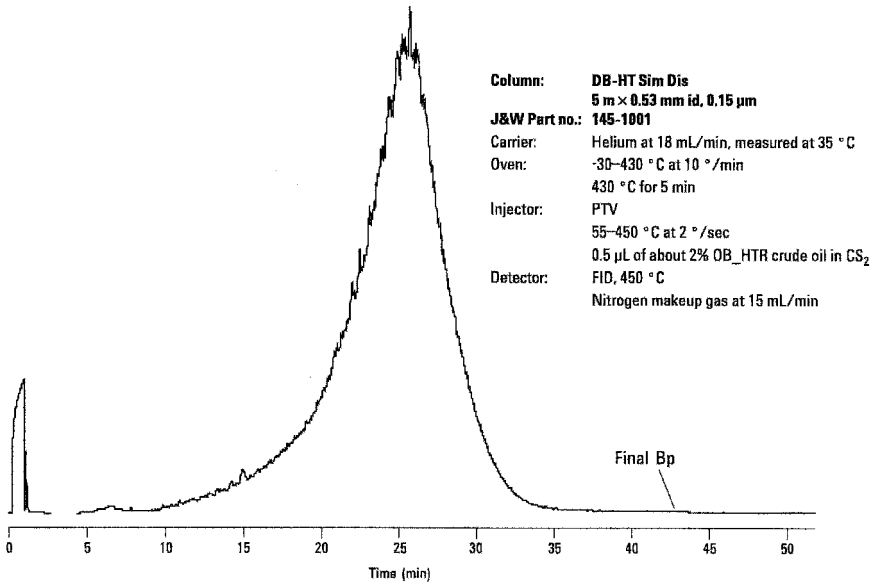
n-paraffin standard showing SimDist results from C_6 to C_{110} on the DB-HT Sim Dis.



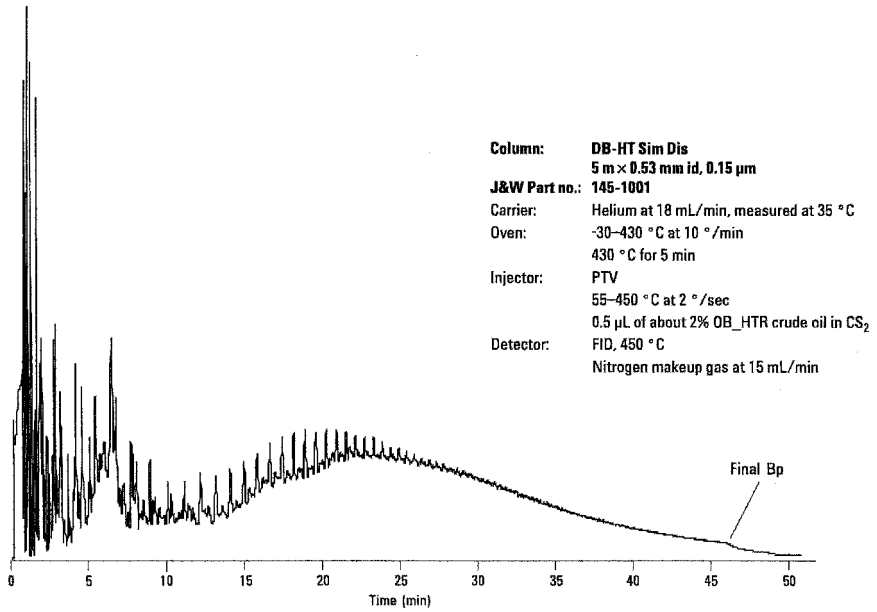
Boiling point vs. retention time for *n*-paraffins on the DB-HT Sim Dis using the conditions shown.

FIGURE 13.23 High temperature simulated distillation standard chromatogram and the resulting calibration. (Reprinted with permission from Reference 72, Agilent Technologies, Inc., Folsom, CA 95630.)

temperatures do not allow the use of fused-silica columns so surface deactivated capillary stainless-steel tubing is required. An example of the *n*-parrafin calibration analysis and calibration is shown in Figure 13.23. Chromatograms of two reference crude oils are shown in Figure 13.24. The cut-point yields obtained by HTGC and distillation methods (D2892/D5236) are very similar, varying by less


Column: DB-HT Sim Dis
5 m × 0.53 mm id, 0.15 µm
J&W Part no.: 145-1001
Carrier: Helium at 18 mL/min, measured at 35 °C
Oven: -30–430 °C at 10 °/min
430 °C for 5 min
Injector: PTV
55–450 °C at 2 °/sec
0.5 µL of about 2% OB_HTR crude oil in CS2
Detector: FID, 450 °C
Nitrogen makeup gas at 15 mL/min
Final Bp
0 5 10 15 20 25 30 35 40 45 50
Time (min)

Simulated distillation of a midrange reference crude oil (HTST_REF).


Column: DB-HT Sim Dis
5 m × 0.53 mm id, 0.15 µm
J&W Part no.: 145-1001
Carrier: Helium at 18 mL/min, measured at 35 °C
Oven: -30–430 °C at 10 °/min
430 °C for 5 min
Injector: PTV
55–450 °C at 2 °/sec
0.5 µL of about 2% OB_HTR crude oil in CS2
Detector: FID, 450 °C
Nitrogen makeup gas at 15 mL/min
Final Bp
0 5 10 15 20 25 30 35 40 45 50
Time (min)

Simulated distillation of a full range reference crude oil (OB_HTR).

FIGURE 13.24 High temperature simulated distillation chromatograms of midrange and full range standard crude oils. (Reprinted with permission from Reference 72, Agilent Technologies, Inc., Folsom, CA 95630.)

than 2 wt%, except for the range ~400–480°C (750–900°F), which corresponds to the crossover between the D2892 reflux column method and the D5236 vacuum pot still method. Systematic errors, however, have been observed relating to the relative elution time of aromatic hydrocarbons using capillary columns with different stationary phases. Raia et al. demonstrated the usefulness of this technique for characterization of solvent extraction of residuum (73).

For the heavier petroleum fractions as well as crude oil, analysis problems are encountered as a result of the presence of solids and materials that will not elute from the column. To account for the total sample, an internal standard mixture is used. For heavy refinery cuts, an internal standard mixture, such as C10–C12 *n*-paraffins, can be used such that they elute before the sample. This allows analysis of the sample in a single run. Because of the complexity of crude oils, there are no sections in the sample chromatogram that are void of sample components. Thus an analysis of the sample with and without internal standard (C14–C17 normal hydrocarbons) is required. Typically, results are reported for these samples up to 538°C (1000°F). Use of a Dexsil 300 column allows for determination of final boiling points up to 600°C. This extends the limit of the analysis to C40–C60 hydrocarbons.

Relatively minor differences do exist, however, between simulated and "true" distillations. Because of the historical dependence on D86 and D1160 for process control and product specifications, efforts have been made to establish more accurate correlations (74). For example, ASTM D86 has inherent sources of imprecision, including column holdup and incomplete condensation. Gas chromatographic distillation cannot measure the initial boiling point in quite the same way as physical distillation. The initial boiling point is the temperature at which the vapor pressure of the bulk sample is equal to the barometric pressure. Also, even very nonpolar column liquid phases exhibit a slight discrimination between alkanes and other hydrocarbon types. This slightly distorts the boiling point curve. However, this becomes significant only with highly naphthenic or aromatic samples.

Stuckey (75) developed calculation response factors that take into account the chemical nature of the sample. The UOP characterization factor is a measure of chemical character and is used along with boiling point to determine the appropriate response factor (76).

Particular care must be taken when performing a simulated distillation on gasoline samples. Although either a TCD or an FID can be used with appropriate response factors, high relative concentrations of some light components such as butane (which can be as high as 10%) could exceed the saturation limit for a FID.

Not withstanding these relatively minor difficulties, simulated gas chromatographic distillation has expanded greatly. The basic precision and ease of automation represent significant cost savings. Furthermore, gas chromatographic distillation data are being used to replace other manual tests (77). Models have been developed to estimate Reid vapor pressure for gasoline with good reliability. Such data are used to predict engine performance and are included in the product specification for gasoline. Engine performance parameters such as starting index,

vapor-lock index, and warmup index can also be calculated from the boiling-range distribution.

13.3.3 Hydrocarbon Type Analysis

Along with boiling-point distribution, refinery streams are normally characterized by a hydrocarbon type analysis. This analysis is used to determine the saturate–olefin–aromatic (SOA) content of a sample. The original approach to this analysis was to separate a sample into three distinct fractions. These fractions were then analyzed for individual components. Another more recent approach has been to utilize high-resolution GC to provide a total analysis. Individual components are then totaled to provide a type analysis. This analysis allows for the determination of PONA (paraffins–olefins–naphthenes–aromatics) or PIANO (if isoparaffins are also resolved). Regardless of the methodology, hydrocarbon type analyses are necessary for valuing feedstocks as well as optimizing reforming and cracking conditions for naphthas and gas oils. They are also important for the characterization of the quality of product fuels, including gasoline. For instance, aromatic content is important for the octane rating of unleaded gasolines and for smoke elimination in jet fuels.

The standard procedure for hydrocarbon type analysis is ASTM D1319, the fluorescent indicator adsorption (FIA) method (19). This method covers determination of petroleum fractions that distill below 600°F (315°C). The sample is separated into three fractions by use of a silica column. A mixture of fluorescent dyes is also added with the sample. The dyes are then separated selectively with the hydrocarbon types. The volume percentage of each hydrocarbon type is calculated from the length of each zone as indicated by the dyes. Further analysis of each of the three fractions has traditionally been performed by mass spectrometry. In addition to the disadvantage of being time-consuming, this column chromatographic analysis cannot determine the C5 and lighter hydrocarbons because of their volatility.

Both liquid and gas chromatographic techniques have been developed to improve this analysis. Suatoni and co-workers (78,79) have performed most of the work by utilizing liquid chromatography. Soulages and Brieva (80) developed a gas chromatographic analysis that relies on selective adsorption of components. The sample is split onto three parallel columns. The saturates content is determined from one column that has a mercuric perchlorate–perchloric acid absorber for olefins and aromatics. The second column is merely a delayer for a portion of the total sample. The third column has a mercuric sulfate–sulfuric acid absorber to retain the olefins. Figure 13.25 is a typical chromatogram from this system.

Since the individual types are determined by difference, the error in the analysis must be considered when one fraction is small relative to the others. A somewhat simpler system that does not rely on trapping of components has been developed by Ury (81). Trapping can cause errors when only small amounts of an individual component are present. An *N*,*N*-bis(2-cyanoethyl)formamide (CEF) column was used to delay the aromatics. The olefins were then separated from

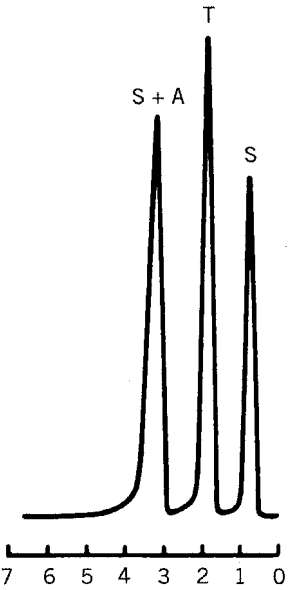


FIGURE 13.25 Chromatogram from hydrocarbon type analysis of gasoline. (Reprinted with permission from Reference 80, *Journal of Chromatographic Science*, Copyright 1971, Preston Publications, A Division of Preston Industries, Inc.)

saturates with a column of cupric sulfate on silicagel. A typical chromatogram for a gasoline sample is shown in Figure 13.26.

Systems have also been developed to give more detailed analyses of the individual compound classes in addition to the basic analysis by type. Stavinoha (82) used a CEF column for preseparation as well as total analysis of the aromatic components. The perchlorate and sulfate absorbers mentioned previously were again used for determination of the saturates and olefins. Mathews et al. (83) utilized dual porous-layer open tubular (PLOT) capillary columns to analyze the aromatics in gasoline and light oils. The first column is loaded with CEF to retain the aromatics. It can also be used alone to give a total saturate–aromatics split. A second column with a mixed liquid phase of di-*n*-propyl tetrachlorophthalate (DPTCP), Carbowax 400, and methylabietate separates the individual aromatics. Figure 13.27 contains chromatograms comparing the aromatics in three commercial gasolines. The ASTM procedures for aromatics in gasolines use either a single- or a dual-column approach. Method D4420, which replaced D2267, is used for determination of benzene, toluene, C8, C9, and heavier aromatics in finished motor gasoline as well as reformate feed and product samples (19). This dual-column, dual-filament TCD system uses a polar column, such as OV-275, to hold up the aromatics and to vent the nonaromatics. This column is then backflushed onto a second nonpolar column, such as SE-30 or OV-101, to separate the aromatics by boiling point. After eluting the C8 aromatics, the C9 and heavier aromatics are backflushed to the detector.

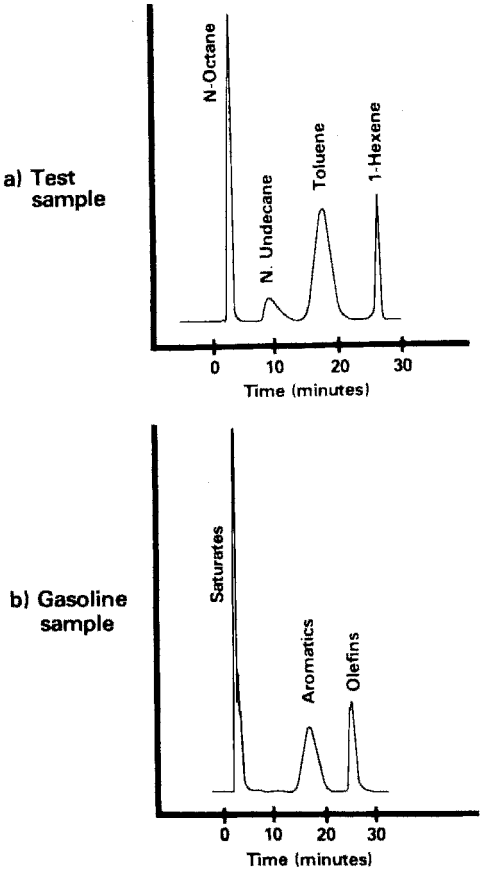


FIGURE 13.26 Chromatogram from hydrocarbon type analysis of a test sample and a typical gasoline sample. (Reprinted from Reference 81, *Journal of Chromatographic Science*, Copyright 1981, Preston Publications, A Division of Preston Industries, Inc.)

Qualitative results are obtained by factors from a calibration blend. This analysis is important to verify the aromatic content due to air-pollution regulations. For benzene and toluene in motor and aviation gasolines a two-column system is specified by method D3606 suggests the use of a methyl silicone column to backflush the heavier components (19). The octane and lighter components are passed on to a polar column such as a 1,2,3,-tris(2-cyanoethoxy)propane column to separate the benzene, toluene, and methyl ethyl ketone internal standard. Ethanol does interfere with this method although Wasson–ECE has developed a column set that eliminates this coelution (84). A typical chromatogram is shown in Figure 13.28. The importance of this test is to monitor benzene levels because of its toxicity. Finally, ASTM D5580 is another two-column system that separates benzene, toluene, xylenes, and C9 and heavier aromatics in 46 min (85).

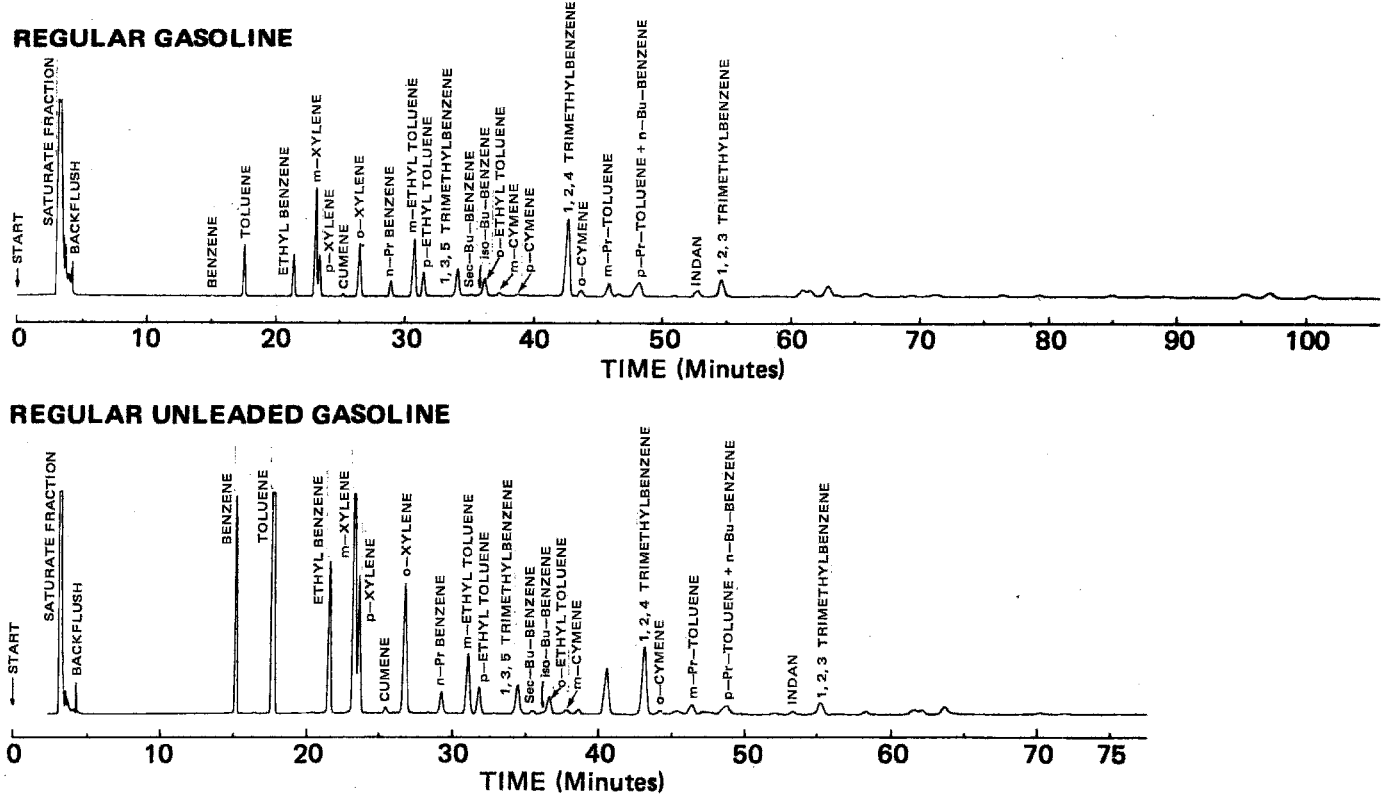


FIGURE 13.27 Two-dimensional analysis of the aromatic fraction of three commercial gasoline samples. (Reprinted with permission from Reference 83, *Journal of Chromatographic Science*, Copyright 1982, Preston Publications, A Division of Preston Industries, Inc.)

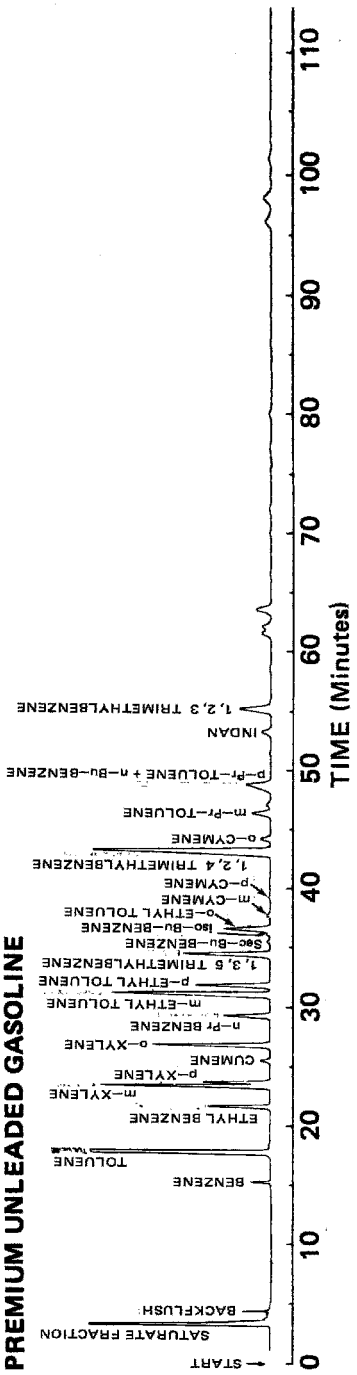


FIGURE 13.27 *(Continued)*

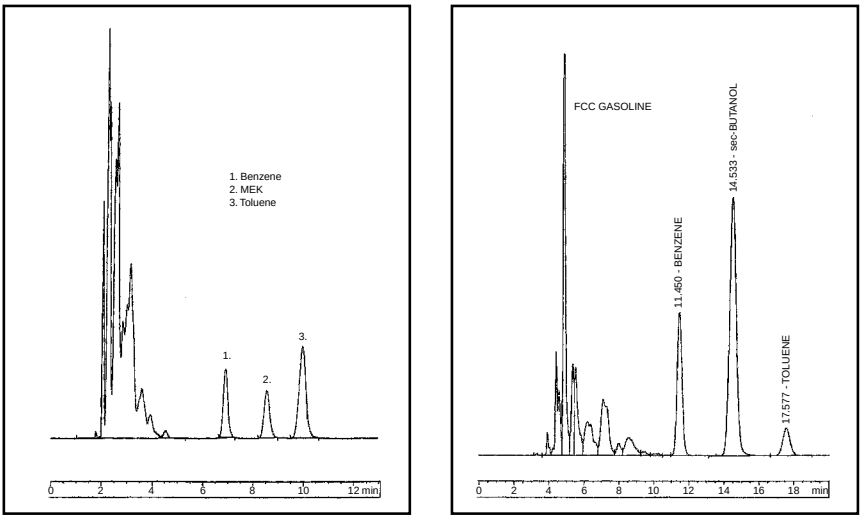


FIGURE 13.28 Typical chromatogram for ASTM D-3606 for benzene and toluene in gasoline. (Reprinted with permission from Reference 84, Wasson-ECE Instrumentation.)

Molecular sieve columns have been used to characterize samples by selectively removing one class of compounds. Folmer (86) and Stuckey (87) utilized this for subtractive chromatography of *n*-paraffins by using parallel columns. In one column, a section of 5A molecular sieve removed the *n*-paraffins, and a SE-52 packed column separated the other components. The other column contained a section of Celite in place of the 5A sieve. With the use of a thermal conductivity detector, a differential signal is obtained for the *n*-paraffins, as shown in Figure 13.29. Mortimer and Luke (88) accomplished the same analysis by first using molecular sieves to remove the *n*-paraffins from vaporized samples. They then destroyed the sieve with hydrofluoric acid, extracted with isooctane, and analyzed the paraffins with a short silicone gum column. A 13X molecular sieve column was used by Garilli et al. (89) for fractions with boiling points up to 185°C. This column separated components by carbon number but partially and irreversibly adsorbed aromatics. As shown in Figure 13.30, they used this analysis to monitor the conversion of a naphtha stream into higher octane paraffins in a platforming (reforming) unit.

A method for a more complete analysis of all of the components was developed by Boer and Van Arkel (90). A three-column system with automatic valve switching and cold traps was utilized to determine paraffins, naphthenes, and aromatics. Thus it is commonly referred to as a *PNA* analysis. A polar column of tris(cyanoethyl)nitromethane was used to retard the aromatics. A 13X molecular sieve column then separated the paraffins and naphthenes. A nonpolar column of UCCW-982 provided further separation of the aromatics and naphthene components. Figure 13.31 shows a typical chromatogram along with an indication of the column switching involved.

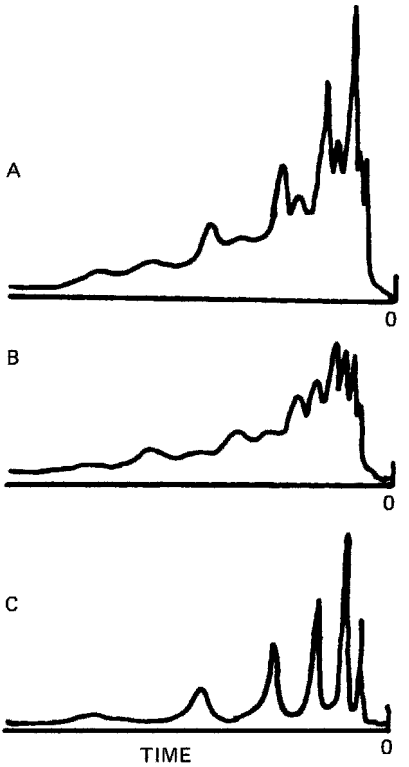


FIGURE 13.29 Substractive chromatographic analysis of a kerosene sample: (A) total sample, (B) nonnormal paraffins, (C) normal paraffins—difference between (A) and (B). (Reprinted with permission from Reference 87, *Analytica Chimica Acta*, Copyright 1972.)

Boer et al. (91) modified their original PNA analyzer to allow analysis of samples with final boiling points above 200°C (up to 275°C). These modifications have minimized problems with cold-trapping fractions by requiring only medium- and high-boiling components to be trapped along with no flow reversal through the trap. The system is basically two chromatographic systems coupled by a switchable cold trap. One system contains the polar OV-275 and the 13X molecular sieve columns in series. The other contains the nonpolar OV-101 column. A Pt/Al_2O_3 hydrogenator has also been added to saturate olefins.

The overall analytical cycle is basically the same as in the original analyzer. The sample is injected on the polar column. The paraffins and mononaphthenes are trapped as they elute onto a 13X molecular sieve column. The low-boiling aromatics and higher-boiling components are eluted from the polar column into a trap. These trapped components are then separated on the nonpolar column while the high-boiling compounds (b.p. >200°C) are backflushed as a single peak. The higher-boiling aromatics are then eluted from the polar column, trapped, and analyzed on the nonpolar column. The highest-boiling aromatics are backflushed

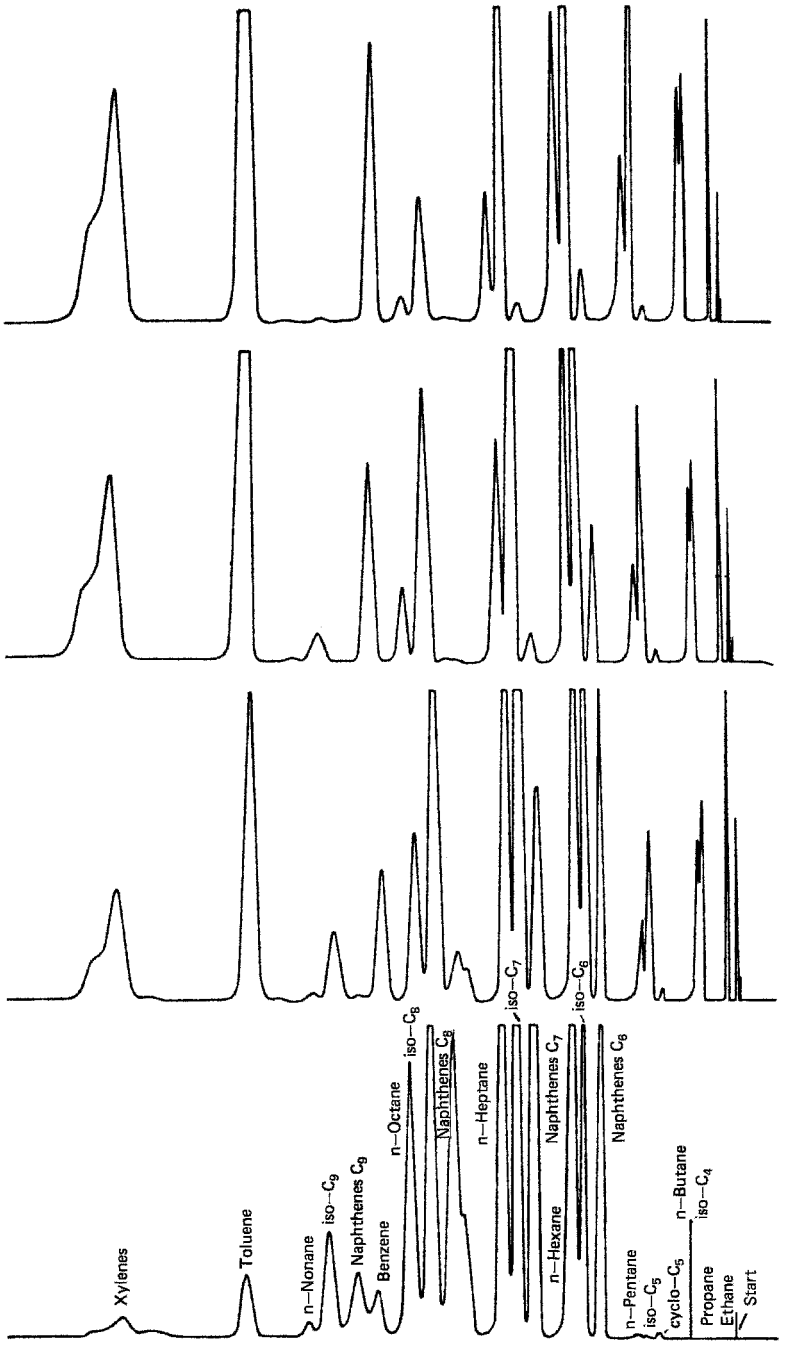


FIGURE 13.30 Four chromatograms, from bottom to top, the first platforming reactor inlet and the outlets from the first, second, and third reactors. (Reprinted with permission from Reference 89, *Journal of Chromatography*, Copyright 1973.)

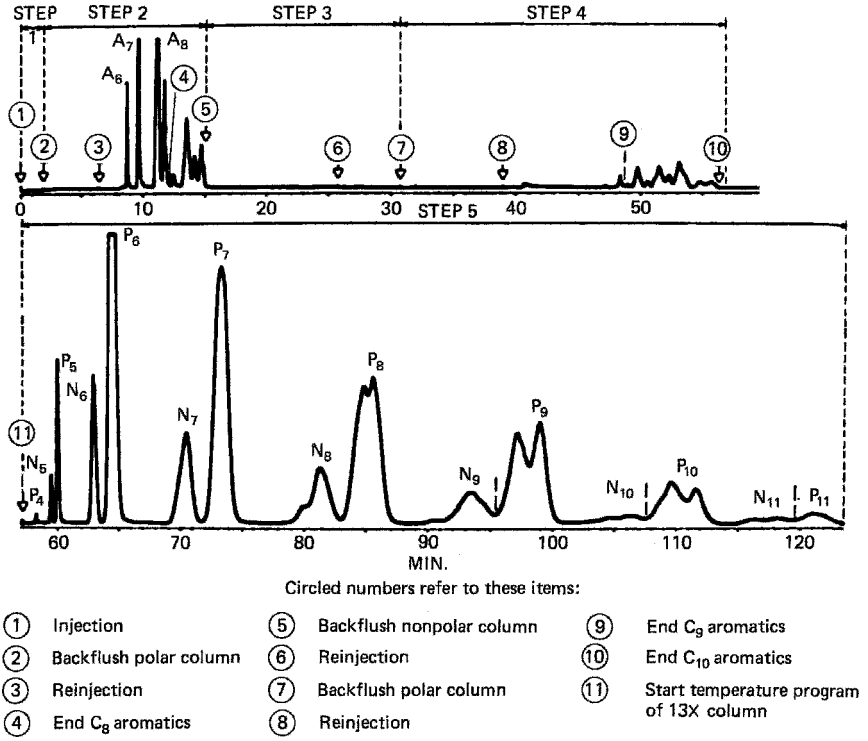


FIGURE 13.31 Typical chromatogram for a heavy naphtha by a PNA analyzer. (Reproduced with permission from Reference 90, *Hydrocarbon Processing*, Feb. 1972.)

from the polar column and then analyzed on the nonpolar columns. The nonpolar column is backflushed following both of the previous cycles, and the final step involves temperature programming.

Forty-seven peaks representing single or multiple components are obtained from this analysis. A single rather than dual FID is used to increase the accuracy and repeatability of this analysis at the expense of doubling the analysis time. The inherent repeatability, accuracy, and reproducibility of this multicolumn technique was evaluated by Van Arkel et al. (92).

In improving this PNA method, Curvers and van der Sluys (93) reduced the analysis time from 2 h to 70 min. They accomplished this through optimization of operating conditions while also improving resolution. By modifying the configuration around the nonpolar OV-101 column, the desorption of aromatics from the Tenax column trap was changed to backflushing rather than foreflushing through the OV-101 column. Also, the carrier-gas flowrate was optimized for the OV-101 column. Finally, nonlinear temperature programming was used for the 13X column.

With this PNA analyzer, olefins can be determined only by dual injections. One analysis is made with hydrogenation of the unsaturates. The other is made after

adsorption of the unsaturates. The aromatics are used as the internal standard, but this technique suffers from inaccuracy. A solution to this problem is the reversible olefins trap developed by Boeren et al. (94). Curvers and van den Engel (95) have utilized this reversible olefins trap with their optimized conditions to provide a PONA analysis in 1.5 h, as shown in Figure 13.32. In this system, the nonaromatics eluting from the OV-275 column are flushed through the olefins trap. The unsaturated components are retained, while the saturates are separated by the 13X column. After the elution of dodecane, the olefins are released with rapid heating of the trap.

With the advances in high-resolution GC, ASTM D5134 provides a hydrocarbon type analysis for gasolines and naphtha using a single column. The HP-PONA column, a 50-m fused-silica capillary column coated with a crosslinked dimethylsiloxane phase, has been developed for this analysis (96). This PONA analysis is capable of resolving individual paraffins, olefins, naphthenes, and aromatics, as shown in Figure 13.33. Analysis of a gasoline sample is shown in Figure 13.34. Supelco's Petrocol DH capillary column is capable of separating more than 300 gasoline components in less than 110 min under ambient initial temperature conditions (71). As shown in Figure 13.34, greater separation of propane and the C4 compounds, *m*- and *p*-xylene, and other light components is possible with this nonpolar column. Each peak is combined into its respective groups. The system is calibrated with a known mixture of 103 compounds. It is optimized for C3–C11 components, and only the lighter olefins are completely resolved. For routine analyses, care must be taken since a slight shift in relative retention times

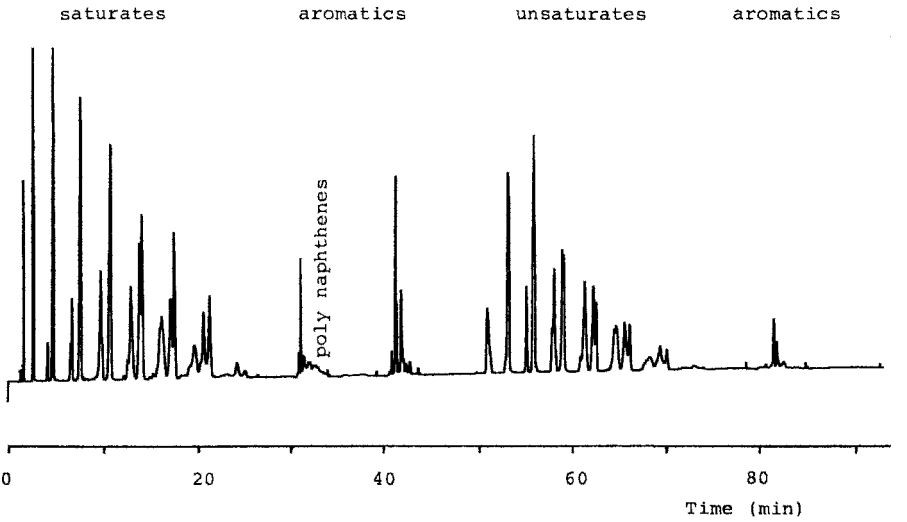


FIGURE 13.32 Typical PONA chromatogram of a visbreaker naphtha. Saturates and unsaturates are separated into naphthalenes and paraffins according to carbon number. (Reproduced with permission from Reference 95, *Journal of Chromatographic Science*, Copyright 1988, Preston Publication, A Division of Preston Industries, Inc.)

0.01
4.61
6.01
6.44
6.55
C5
7.02
8.09
9.63
9.43
10.62
C6
11.64
12.30
12.50
13.62
14.21
14.52
15.47
16.46
17.43
18.10
18.42
18.75
20.40
C7
22.78
23.13
24.21
24.69
25.39
26.74
26.36
27.24

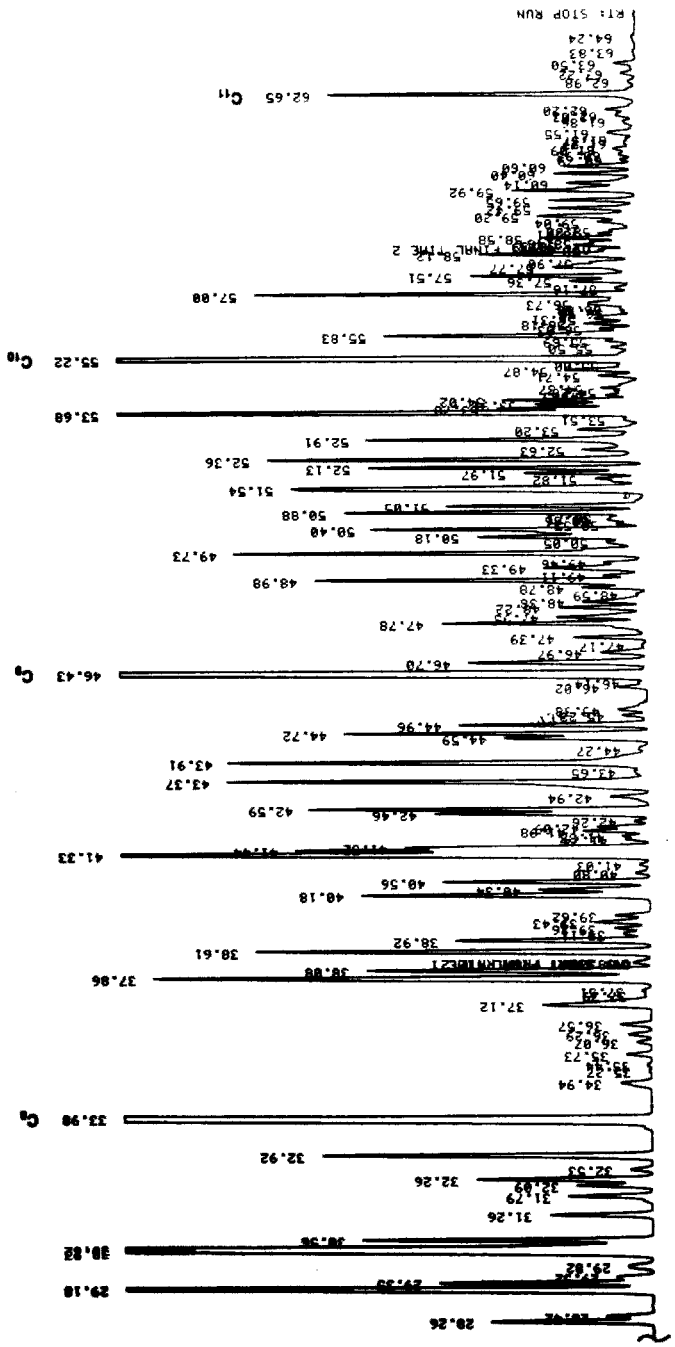


FIGURE 13.33 Typical chromatogram for PONA analysis of a naphtha sample. (Reprinted with permission from Reference 96, Hewlett–Packard Co.)

will dramatically affect the results. Even without full calibration, this method is somewhat easier and more informative than a typical hydrocarbon type analysis. This is due mainly to its capability to determine relative differences in plant process samples.

With the complexity of the more than 230 components in samples such as gasoline, positive identification by retention time is often difficult. Even with

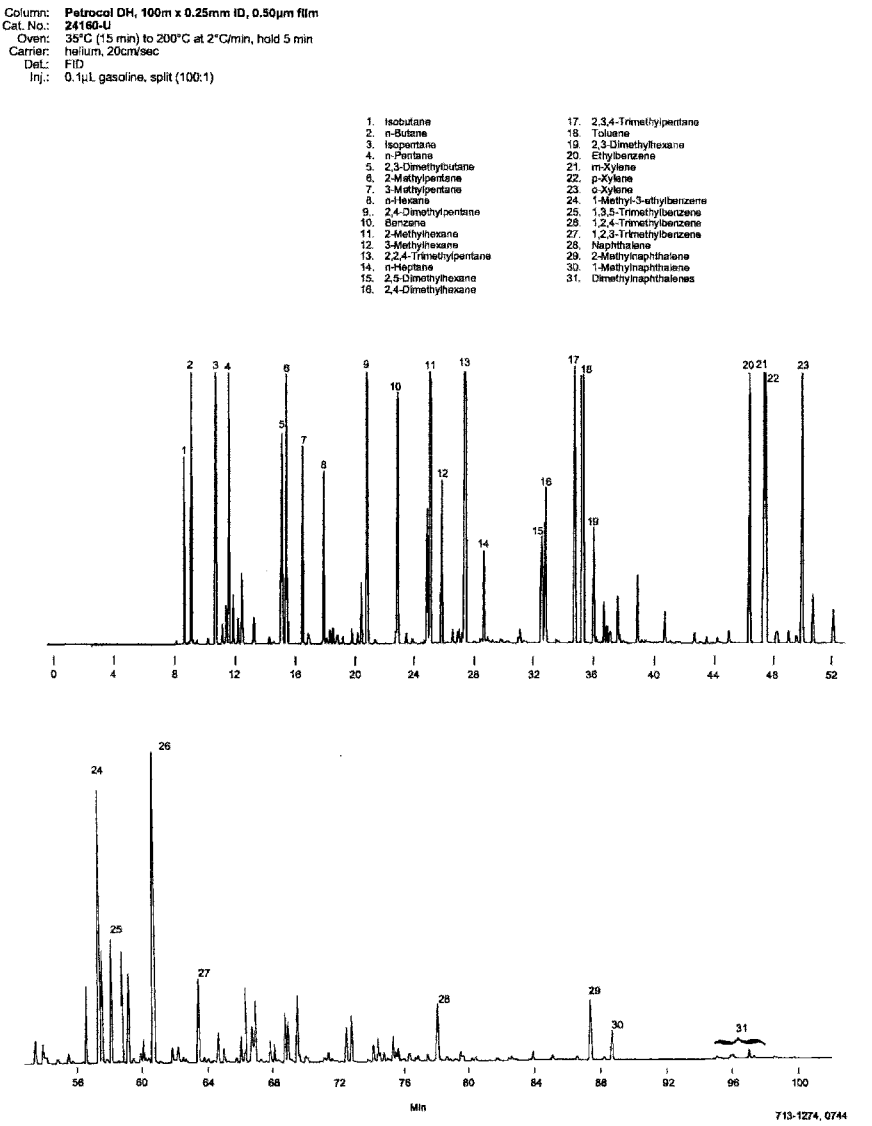


FIGURE 13.34 High-resolution gas chromatogram of a premium unleaded gasoline. (Reprinted with permission from Supelco, Inc, Bellefonte, PA 16823.)

a mass spectroscopy detector, identification of overlapping peaks is not always possible, especially for monoolefins and naphthenes, which have the same molecular weight. This problem has been alleviated by Shimoni et al. (97), who utilized precolumn sulfonation to trap olefins and aromatic compounds. The precolumn is a glass injection port packed with a small amount of solid support impregnated with sulfuric acid followed by an alkaline trap. This trapping allows for easy discrimination of saturated and unsaturated hydrocarbons.

A comparison of the PONA techniques including the classical FIA method, the multicolumn–trap–GC method, and the high-resolution capillary GC method was made by Kosal et al. (98). Capillary GC has the advantage in that it gives information on individual components. However, peak identification must be checked carefully, especially for different types of samples. It is best suited for laboratories dealing with the same type of samples that have slight variations in individual components. Separation of higher boiling components can also be a problem. The multicolumn/trap GC, on the other hand, is much more complicated, especially with maintenance of the switching valves. Finally, the classical FIA method provides only limited information with no individual component analysis or carbon number distribution. It also requires longer analysis time and more operator attention.

To monitor the catalytic conversion of methanol into gasoline, a PONA type analysis was developed by Bloch et al. (99). The catalytic process to convert methanol into gasoline produces a mixture of hydrocarbons with a maximum carbon number of 11. A complete analysis of these hydrocarbons was accomplished by using high-resolution columns along with aromatic precutting and olefin adsorption. A computer was used for programming of column temperature, flow, and valve switching. The computer also handled the data from three FIDs. An OV-275 WCOT column was used for precutting of the aromatics that were resolved on a squalene SCOT column. The paraffins, naphthenes, and olefins were separated on a squalene SCOT column. The effluent from this column was then split, with part going directly to a FID. The remainder was passed through a mercuric sulfate–sulfuric acid adsorber to remove the olefins and then onto the third FID. With this rather complex system, approximately 200 individual or combinations of compounds were identified and quantified.

Table 13.2 provides a comparison of petroleum reformates and methanol-derived gasolines as determined by this analysis. The unleaded octane numbers for these samples are approximately equal. It can be seen from these data that the gasohol has higher olefin and naphthene content as well as a higher ratio of iso-/*n*-paraffins. Comparison of these data with FIA analysis (ASTM D1319) revealed higher aromatic and lower saturate values along with excellent agreement on the olefins.

13.3.4 Sulfur and Nitrogen Compounds

Because of the unavailability of low-sulfur crude oils, heavy asphaltic crudes and synthetic crudes are now being utilized. These less desirable crudes contain

TABLE 13.2 Comparison of C6+ Hydrocarbon Types in Reformates and Methanol-Derived Gasoline as Determined by Open Tubular Column Selective Olefin Absorption GC

Process Unit Charge Stock	C6 (360°F) Mid-Continent Naphtha	C6 (365°F) Nigerian Naphtha	Methanol
Octane number	97.7	99.2	98.2
Isohexanes	10.25	8.55	12.03
n-Hexane	4.61	3.86	0.60
Hexenes	0.10	0.10	1.98
Isoheptanes	7.37	5.89	5.14
n-Heptane	2.10	1.71	0.31
Heptenes	0.10	0.10	2.37
Isooctanes	2.57	2.50	1.05
n-Octane	0.64	0.50	0.06
Octenes	—	—	3.27
C9+ (P+O+N_t)[a]	0.46	0.91	2.20
Naphthenes (C6–C8)	1.23	1.67	9.10
Benzene	6.09	6.58	0.29
Toluene	22.04	20.40	2.96
C8 Aromatics	25.24	23.43	18.40
C9 Aromatics	14.20	17.72	25.26
C10 Aromatics	1.59	4.17	11.97
C11+ Aromatics	1.41	1.91	3.01
Paraffin total[b]	28.00	23.92	21.39
Olefin total	0.20	0.20	7.62
Naphthene total	1.23	−1.67	9.10
Aromatic total	70.57	74.21	61.89

[a] N_t is total cyclopentanes and cyclohexanes.
[b] Includes C9+ (P+O+N_t).

Source: Reprinted with permission from Reference 99, *Journal of Chromatographic Science*, Copyright 1977, Preston Publications, Inc.

heterocyclic sulfur and nitrogen compounds. The sulfur and nitrogen compounds, however, are catalyst poisons and must be removed before they are refined. Their removal is also necessary for the performance, storage stability, and general acceptability of final petroleum products.

For monitoring of process streams and product quality, determination of total and individual sulfur compounds is required down to ppm levels. The characterization of individual compounds has involved primarily the combination of GC using packed columns and element-selective detectors. For sulfur compounds, Druschel (100) utilized a Carbowax 20 M column with a microcoulometric detector. A combustion tube at 550°C converts the sulfur compounds into SO_2 as they elute from the column. The SO_2 is then titrated by electrogenerated iodine. A typical application for this analysis is shown in Figure 13.35. It was used to monitor the removal of condensed thiophenes by hydrotreating of

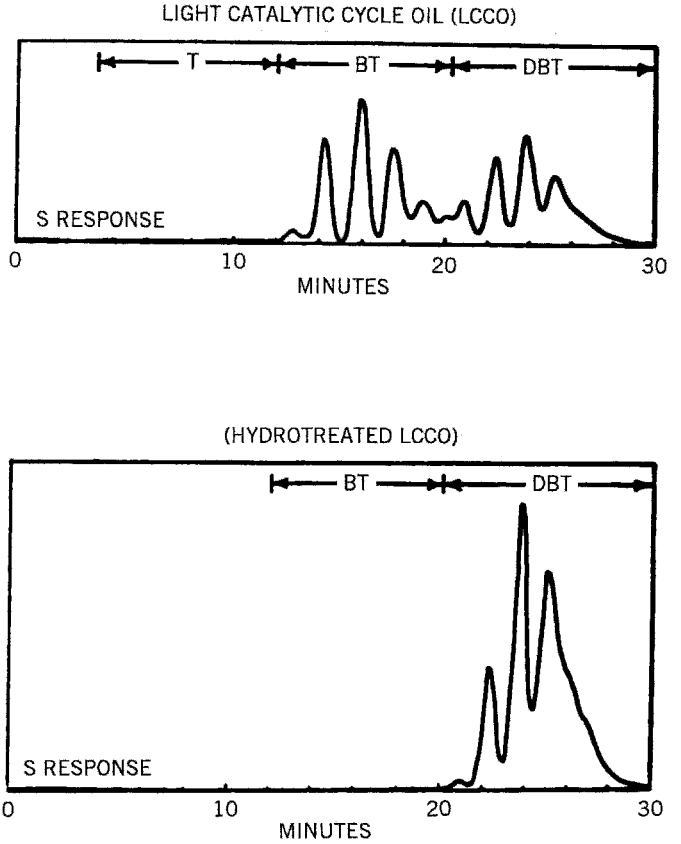


FIGURE 13.35 Gas chromatographic–microcoulometric chromatograms of light catalytic cycle oil (LCCO) demonstrating the removal of benzotheophenes by hydrotreating. (Reproduced with permission of the author, Reference 100.)

light catalytic cycle oil (LCCO). Although this analysis has low resolution, it is highly selective.

Martin (101) and Albert (102) have similarly determined nitrogen compounds. Hydrogenolysis is used to convert the nitrogen compounds to ammonia. The ammonia is then determined in a microcoulometric cell by generation of hydrogen ions. A typical chromatogram for LCCO using a column with a 12,000-MW (molecular weight) polyethylene liquid phase is shown in Figure 13.35. Like the sulfur analysis, this method has low resolution but high selectivity as long as a scrubber is used to remove the HCl from chlorine compounds.

A selective thermionic specific detector (TSD) was developed by Albert (107) but it is more commonly referred to as a *nitrogen–phosphorous detector* (NPD). It is basically an alkali FID. Figure 13.36 compares the TSD and the microcoulometric detector. More resolution is obtained through the TSD with elimination of the mixing in the transfer line, reactor tube, and titration cell of the

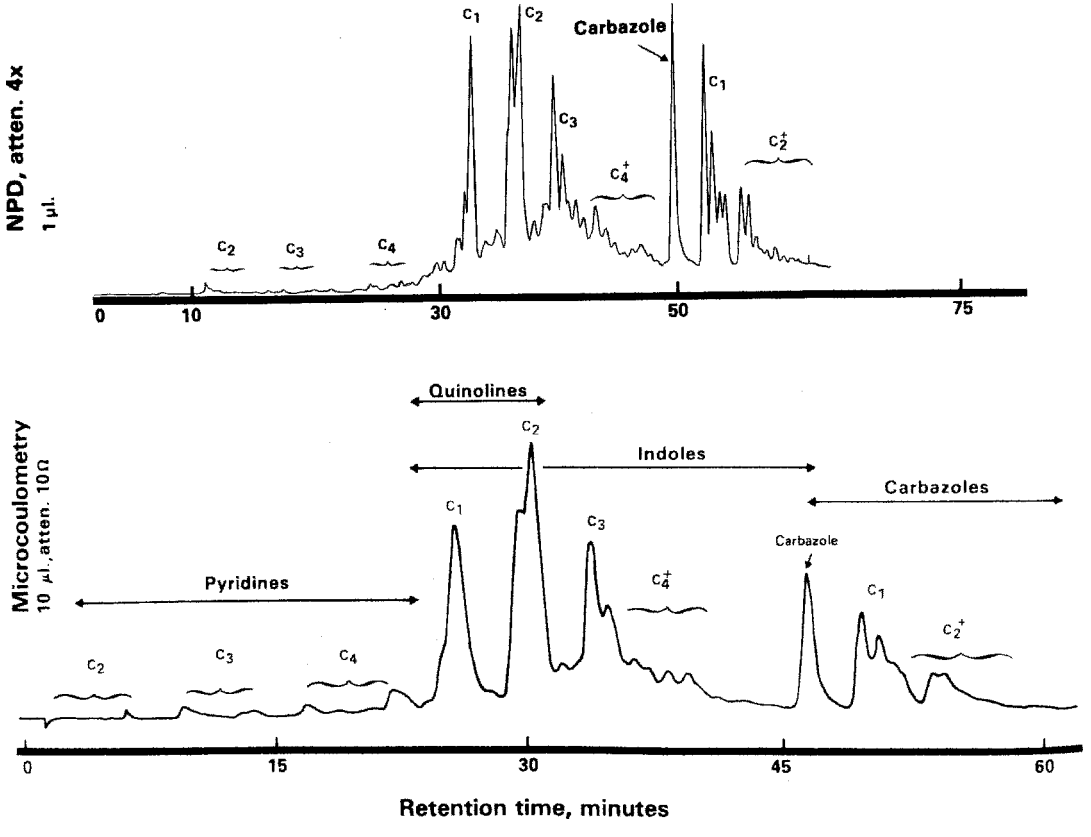


FIGURE 13.36 Comparison of NPD and microcoulometric detector chromatograms of light catalytic cycle oil (LCCO). (Reprinted with permission from Reference 103, *Analytical Chemistry*, Copyright 1978, American Chemical Society.)

microcoulometer. A parallel FID can also be used to monitor the overall boiling point distribution of the sample.

To achieve high resolution of all the heterocyclic compounds, Druschel (104) has utilized fused-silica capillary columns with an SE-54 coating. A flame photometric detector (FPD) was used for sulfur and a TSD was used for nitrogen compounds. Figure 13.37 shows the effect of hydrotreating LCCO. As in Figure 13.35, elimination of the peaks corresponding to the benzothiophenes is obvious. With the high resolution of the capillary column, many of the individual isomers are resolved. Comparison of the chromatograms in Figure 13.37 indicates that certain isomers of the remaining substituted dibenzothiophenes were easier to remove than others. This information allows for a more complete evaluation of catalyst activity.

High resolution of the nitrogen compounds was obtained with a TSD. Figure 13.38 shows the distribution of the individual two- and three-ring nitrogen compounds in LCCO. The resolution in these chromatograms is more obvious when they are compared to Figure 13.36. The effects of hydrotreating are illustrated in Figure 13.38. The two-ring compounds, primarily indoles, were denitrogenated. The high resolution allows identification of several hydrogenated intermediates that were formed without nitrogen removal. This is because hydrogenation is required before nitrogen removal can occur.

An effluent splitter allows for simultaneous detection with the FID and either the FPD or TSD. Besides determination of the overall hydrocarbon distribution, the FID is of value in monitoring for quenching of the FPD. The presence of large background hydrocarbon levels will quench the emission from the sulfur compounds. The results are not quantitative if this occurs. Simultaneous detection with the FID also gives an indication of high levels of components, which may cause a false response by the TSD.

Chemiluminescence detectors show no interference from hydrocarbon quenching (105). They also provide ppb-level sensitivity and response linearity over a large range. With their equimolar response to sulfur and nitrogen regardless of compound type, they allow accurate determination of total sulfur and total nitrogen without the necessity for identification of all components. Figure 13.39 illustrates the determination of sulfur and nitrogen in gasoline and diesel. A GC can be outfitted with both sulfur and nitrogen chemiluminescence detectors as well as an FID (106). A large 14-in. furnace is used to complete combustion while enhancing sensitivity and equimolarity. Also, both sulfur and nitrogen can be determined with the use of an AED (atomic emission detector) which has comparable detection limits and repeatability (107). ASTM D5623-94 allows for both of these detectors for sulfur (19).

13.3.5 Gasoline Additives

In 1991, federal regulations mandated that oxygenated compounds such as alcohols and ethers be used as octane improvers for gasoline. They are intrinsically high-octane components that reduce CO and ozone emissions, while they contain

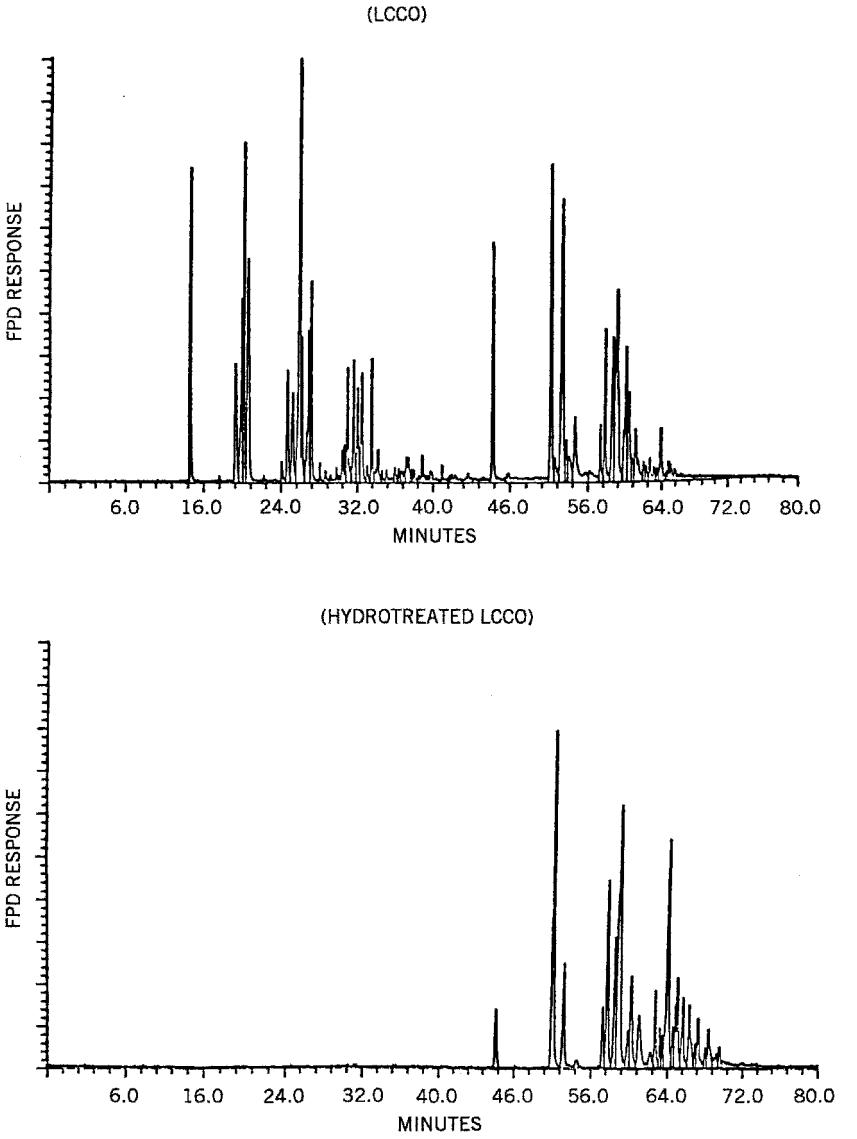


FIGURE 13.37 High-resolution flame photometric detector (FPD) chromatogram of light cycle gas oil (LCCO) demonstrating the effect of hydrotreating on condensed thiophenes. (Reproduced with permission of the author, Reference 104.)

no environmentally objectionable heteroatoms like the previously used metal alkyls, particularly tetraethyl lead. Methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), and *tert*-amyl methyl ether (TAME) were the most commonly used additives. MTBE was the lowest cost of these but is being discontinued because of environmental concerns with groundwater contamination. Ethanol

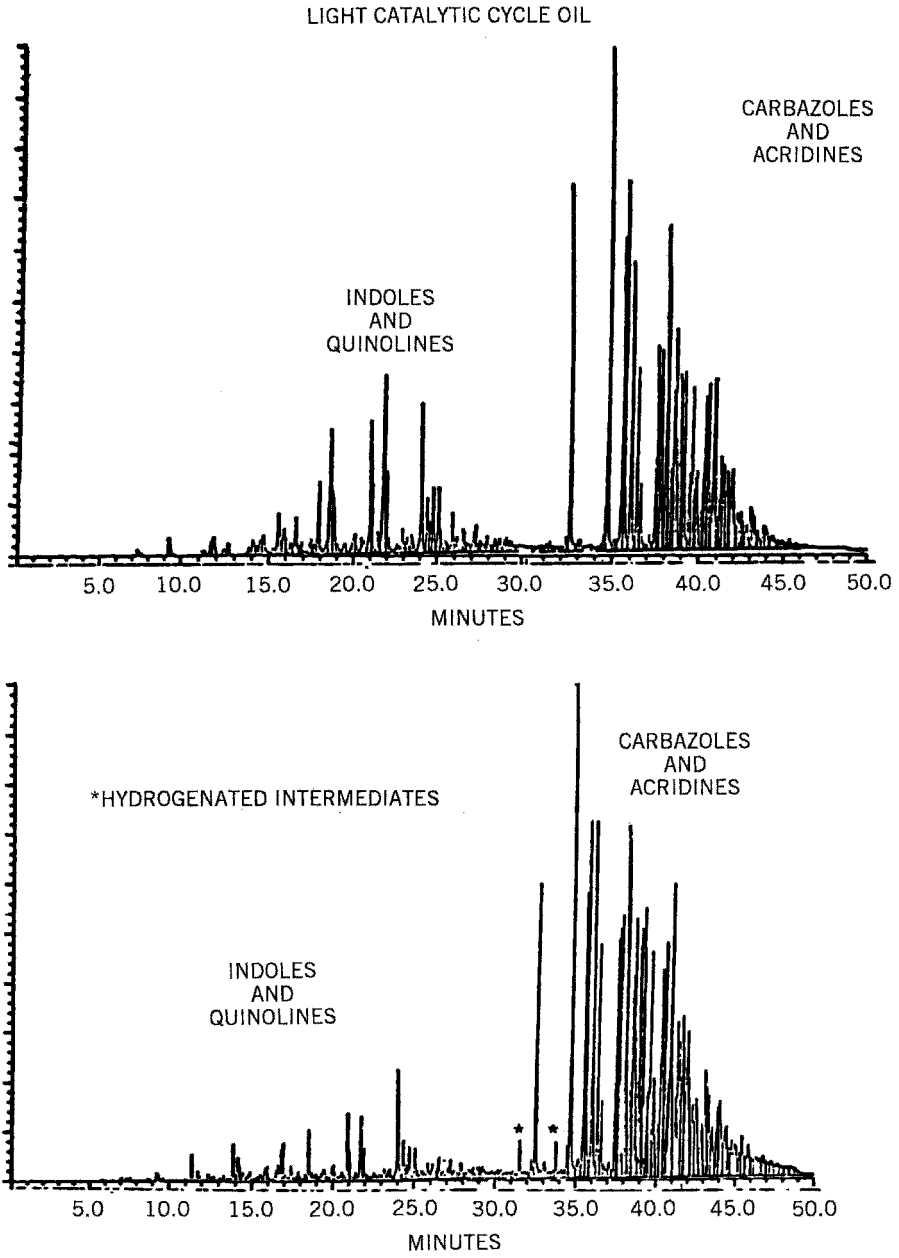


FIGURE 13.38 High-resolution thermionic specific detection (TSD) of light cycle gas oil (LCCO) demonstrating the removal of nitrogen compounds and the formation of hydrogenated intermediates due to hydrotreating. (Reproduced with permission of the author, Reference 104.)

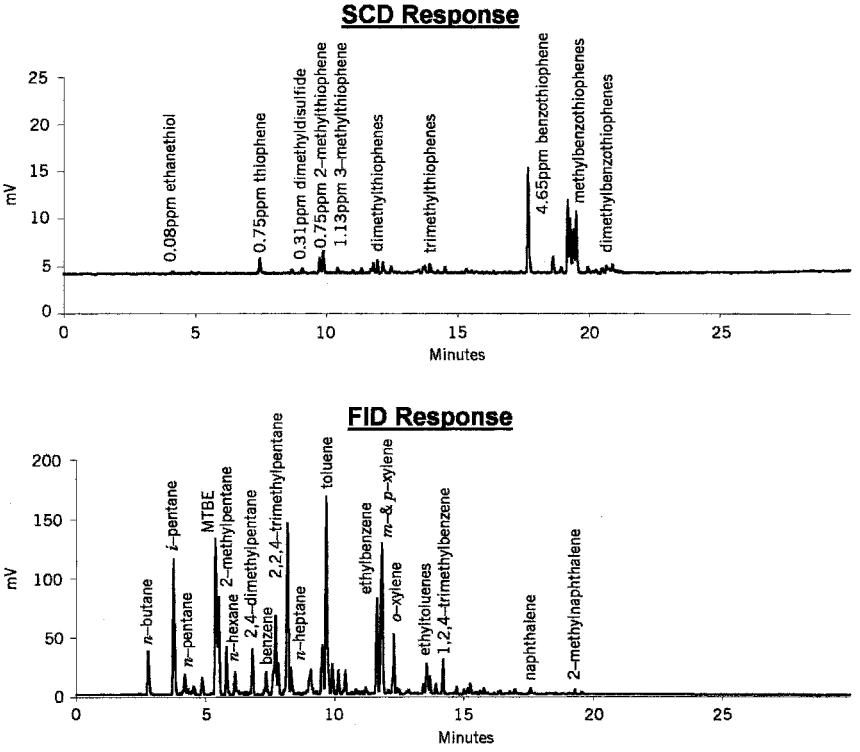


FIGURE 13.39 Comparison of chemiluminescence detector response with an FID on a heavy diesel sample and a regular unleaded gasoline sample. (Reproduced with permission from Reference 106, ANTEK Instruments, Inc.)

is now being used. Because of the complexity of oxygenate-containing samples, a multidimensional gas chromatograph was developed by Naizhong and Green (108) for the analysis of these additives. Their approach has become the basis for the ASTM D4815 method for C1–C4 alcohols and MTBE in gasoline (19). Unfortunately, this method determines a limited range of alcohols and ethers, particularly with the *tert*-amyl alcohol internal standard. Work is currently in progress to expand the method to C1–C5 alcohols, MTBE, TAME, ETBE, and DIPE [di(isopropyl ether)]. Reproducibility is being improved, and the range is being extended to 12 vol% for alcohols and 20% for ethers. The internal standard is also being changed to 1,2-dimethoxyethane (DME). A typical chromatogram is shown in Figure 13.40 (109).

The EPA has also endorsed the use of the oxygen-specific flame ionization detector (O-FID). The O-FID oxygenates analyzer (110) utilizes a single capillary column connected to two microreactors and an FID. A cracking reactor converts any oxygenate into carbon monoxide, which is then catalytically hydrogenated to methane for detection by the FID. The comparison in Figure 13.41 of the standard FID and O-FID for a gasoline sample demonstrates a very selective oxygenates

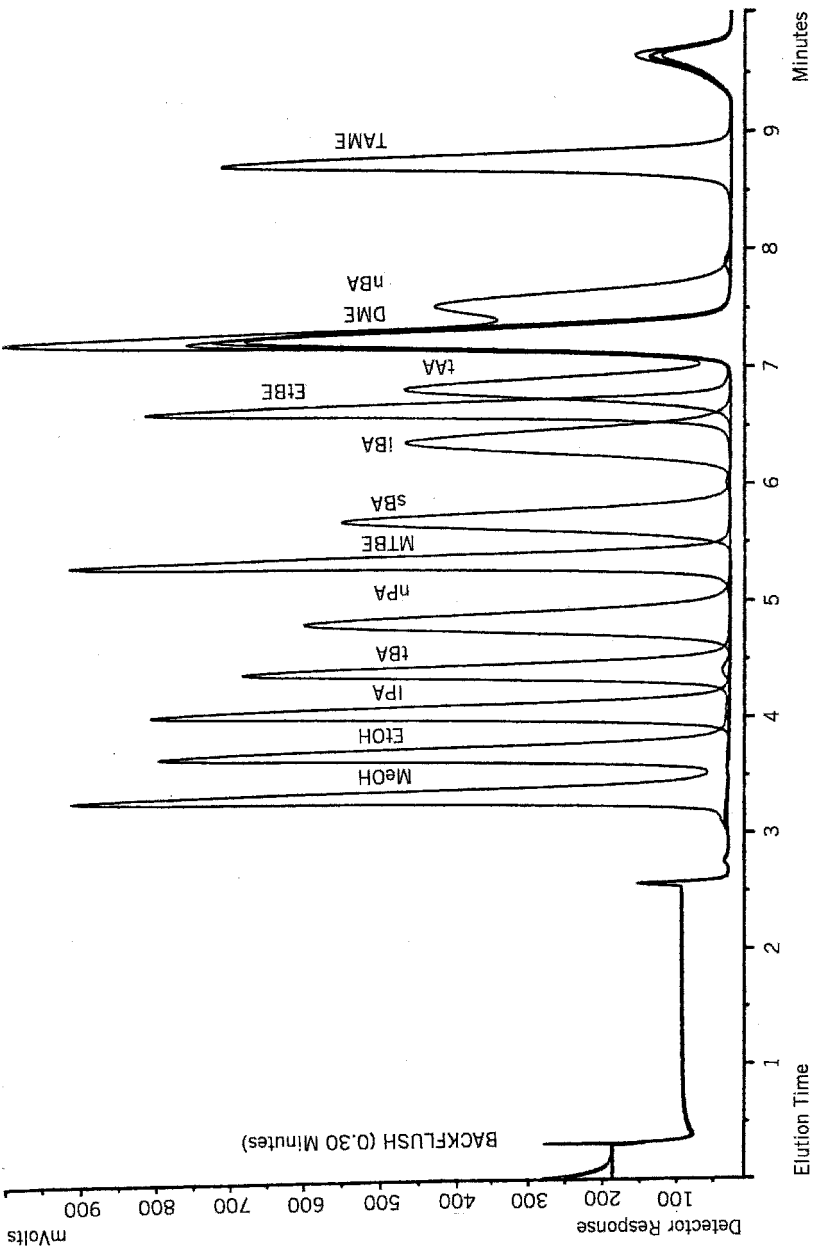


FIGURE 13.40 Composite chromatogram of oxygenates in gasoline range naphtha. (Reproduced with permission of the author, Reference 109.)

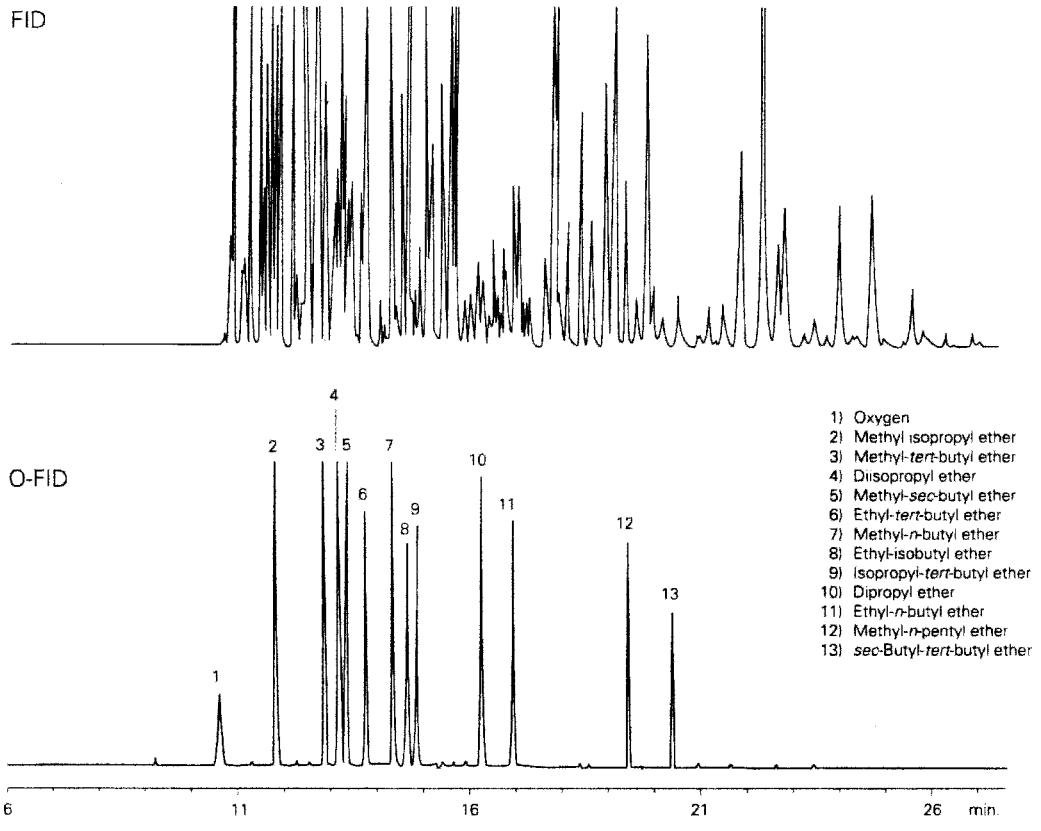


FIGURE 13.41 Determination of a complex mixture of ethers in gasoline on a polar capillary column with a standard FID and the O-FID. [Reproduced from Reference 110 courtesy of Fisons Instruments SpA, Rodano (MI), Italy.]

analysis that is not quenched or suppressed by the sample matrix. The dynamic range of this analysis is 10^5, and 1-propanol is used as an internal standard.

For monitoring of process streams including MTBE process units, a single-column method is more desirable. Rather than use multiple injections, Spock (111) has proposed a 100-m Petrocol DH column. As shown in Figure 13.42, the oxygenates as well as the other hydrocarbons can be quantified using response factors rather than internal or external standards. This method also eliminates the risk of error due to leaking valves and inaccurate valve switch timing in multidimensional GC. However, multiple injections and standardization are required for levels below 100 ppm.

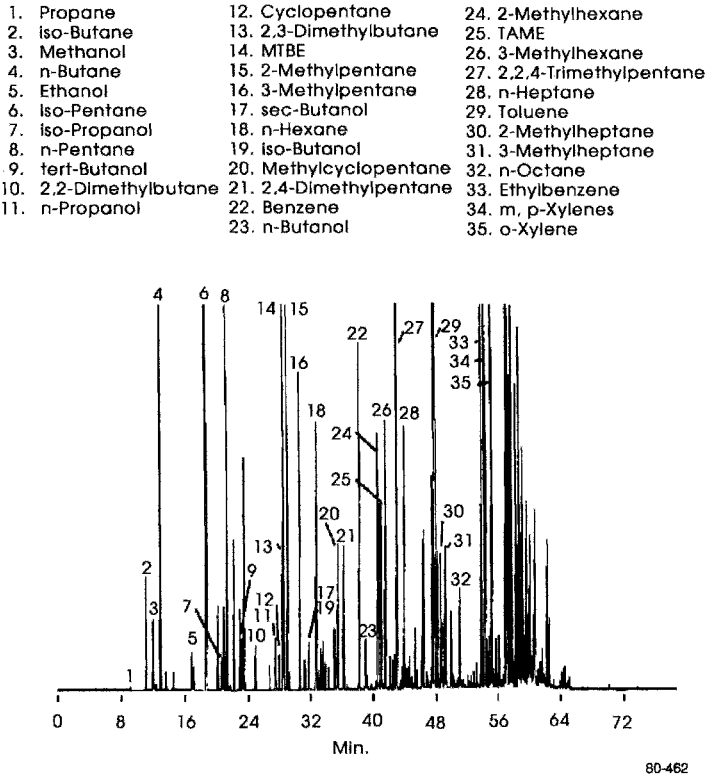


FIGURE 13.42 Oxygenates in unleaded gasoline using a Petrocol DH column, 100 m × 0.25 mm, 0.5-µm film, column temperature: −20 to 65°C at 2°C/min, then to 225°C at 25°C/min. (Reprinted with permission from Reference 111, Supelco, Inc., Bellefonte, PA 16823.)

13.4 PETROCHEMICALS

The term "petrochemicals" refers to the basic chemicals that are derived from refinery petroleum cuts. They are produced by separation of the byproducts from the cracking (pyrolysis) of hydrocarbon streams. These streams range from natural gas to the heavy distillate (gas oil) cuts from a refinery primary fractionator. Some chemicals, such as the aromatics, are separated from various refinery streams. Figure 13.43 is simplified schematic of a petrochemical process.

The basic petrochemicals, which are produced in the largest volumes, are separated into two classes: olefins and aromatics. The olefins include ethylene, propylene, and 1,3-butadiene. The aromatics are benzene and the xylenes. These chemicals are used primarily for the manufacturing of plastics, synthetic rubbers, and fibers. A wide range of other chemicals is also produced in somewhat lesser volumes, but with a variety of applications. This discussion is limited to the major chemicals identified previously.

Because of the large volumes involved and the interchange of products among companies, analyses of all the major petrochemicals are covered by ASTM standards (19). Although other analyses are often used for process control, the ASTM methods are used to resolve discrepancies between laboratories. However, strict adherence to each producer's product specifications often requires modifications to ASTM methods. The applicable ASTM procedures are discussed here along with each petrochemical.

13.4.1 Olefins

The primary purpose of the cracking of natural gas or a wide range of petroleum-derived streams is the production of ethylene. All the other olefins that form are considered to be byproducts. Cracking feedstocks range from natural gas to heavy distillates, depending on their price and the desired products. Originally, the low price of crude oil made petroleum streams, mainly the heavy distillate or gas oils, the most economical. The preferred feedstock for olefin production varies according to economic conditions or pricing of feeds and/or products. Besides profitability, the only other consideration in choosing feedstocks is that the heavier feeds produce more of the heavier olefins, especially butylenes.

Cracking of hydrocarbons to produce olefins can be done thermally or catalytically. *Thermal cracking* is the most typical method. It is basically a pyrolysis step, as discussed in the section on refining. To avoid polymerization reactions with the olefins, steam can be injected to quench these side reactions. This process is commonly referred to as *steam cracking*.

With the variety of feedstocks involved, there is a need to optimize cracking conditions on the basis of an analysis of the feedstock. For refinery distillate cuts, characterization is a difficult analytical problem. A hydrocarbon type analysis, as discussed in Section 13.3.4, can be correlated with cracking yields. Another technique was described by Greco (112) in which microscale pyrolysis was used to simulate cracking. A tube-type pyrolyzer with a quartz

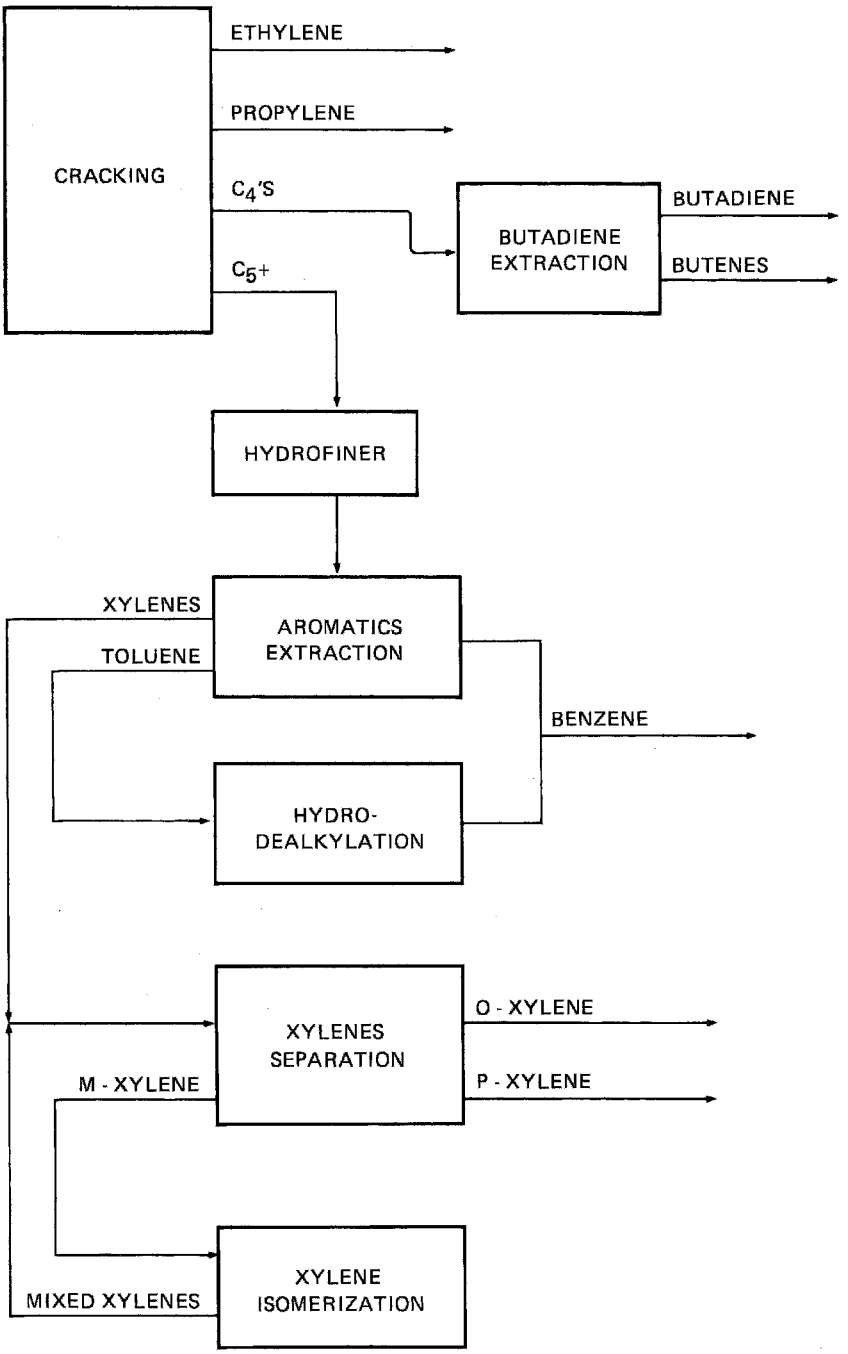


FIGURE 13.43 Simplified schematic of a petrochemical process.

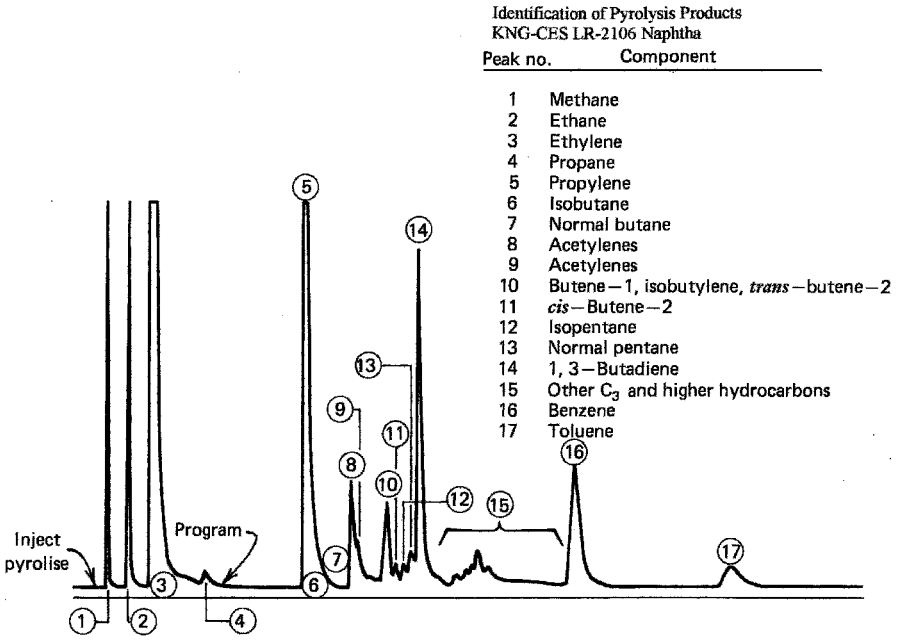


FIGURE 13.44 Chromatogram from analysis of bench-scale pyrolysis of a naphtha sample. (Reprinted with permission from Reference 112, *Journal of Chromatographic Science*, Copyright 1978, Preston Publications, A Division of Preston Industries, Inc.)

insert was coupled to a gas chromatograph. The pyrolysate was separated and determined with a Carbowax 20 M on alumina column and an FID. With this system, a linear relationship was established between the microscale pyrolyzer and commercial cracker yields. Figure 13.44 is a typical chromatogram for a light naphtha. A plot of product distribution as a function of temperature is shown in Figure 13.45. From this plot, an optimum operating temperature can be selected to achieve the desired cracking products. It should be noted, however, that regardless of the technique, exact prediction of cracking yields is extremely difficult.

Analysis of the effluent from a steam cracker is difficult in terms of both sampling and analysis. Because of the wide range of components present, condensation of the heavier compounds must be considered. Heated sample lines as well as heating of the sample bomb are required. Analysis of these components was accomplished by Jordan et al. (113), using a mixed-liquid phase of SE-30 and triscyanopropane column with subambient temperature programming. Because of the similarity, refinery gas analyses are now typically utilized for this analysis. As mentioned in Section 13.3.1, the refinery gas analysis systems are well suited for this application. This includes the permanent gases, of which hydrogen is the most significant for analyzing cracker performance.

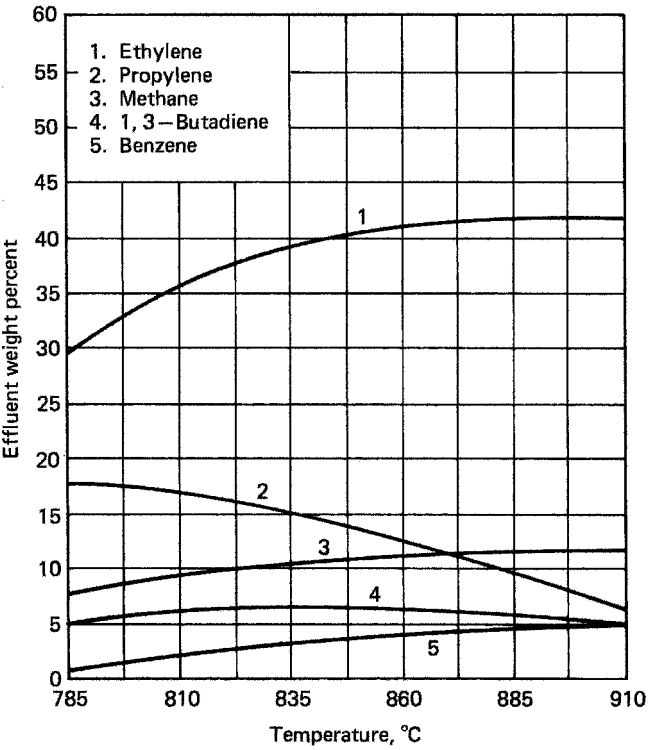


FIGURE 13.45 Plot of product distribution of selected hydrocarbons as a function of temperature for bench-scale pyrolysis of a naphtha sample. (Reprinted with permission from Reference 112, *Journal of Chromatographic Science*, Copyright 1978, Preston Publications, A Division of Preston Industries, Inc.)

13.4.1.1 Ethylene

Ethylene is produced by fractionation of the steam cracker effluent. In order to monitor this process as well as product quality, many analyses have been developed. ASTM methods D2504, D2505, and D6159 are for analysis of high-purity ethylene. ASTM D2504 is used for determination of hydrogen, nitrogen, oxygen, and carbon monoxide and utilizes three different packed-column systems (19). ASTM D2505 is used for determination of carbon dioxide, methane, ethane, acetylene, and other hydrocarbons. Methane and ethane are determined by use of a silicagel column. Acetylene is analyzed by using a hexadecane column in series with a squalene column. A hexamethylphosphoramide column is used to determine propylene and heavier impurities. Carbon dioxide, the final component, is determined by using a column of activated charcoal that has been impregnated with a solution of silver nitrate and β,β′-oxydipropionitrile. Besides the difficulties associated with multiple analyses, this ASTM method does not achieve baseline resolution of the acetylene and propylene.

An analysis for all the C2 and lighter impurities in ethylene has been reported by Zlatkis and Kaufman (114). A Porapak Q column was used with a TC detector for the overall analysis (Figure 13.46). An FID was used for determination of trace acetylene levels. Those authors also used a carbon molecular sieve (Carbosieve) column for this analysis (115). With the use of short packed capillary columns, this analysis can be completed in less than 10 ms. It was subsequently demonstrated by Supelco (116) that the C_3 hydrocarbons could also be separated with a Carbosieve column.

An analyzer has been developed by Wasson–ECE (117) for an extensive analysis of the impurities in ethylene. This is a dual automated multicolumn system that utilizes parallel injections. An FID is used to determine trace levels of hydrocarbon impurities, as shown in Figure 13.47a. To monitor CO and CO_2, they are separated, converted to methane using a methanizer, and then detected with the FID. Additionally, this application is capable of monitoring methanol with a detection limit of 20 ppb, as shown in Figure 13.47b. Methanol is important because it is used for deicing ethylene systems.

One interesting and very significant analytical problem in the operation of an ethylene plant is the control of acetylene along with the other impurities in the process. The tower system is set up with a deethanizer to first separate all the C2 hydrocarbons from the C3 and heavier components. The C2 hydrocarbons taken overhead in this tower contain up to 2% acetylene. This stream is then

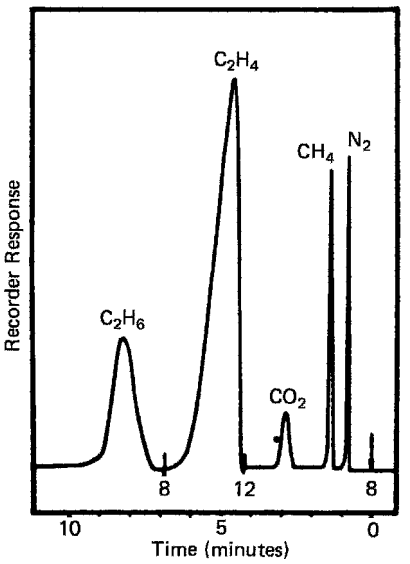


FIGURE 13.46 Chromatogram of ethylene and ppm levels of impurities. (Reprinted with permission from Reference 114, *Journal of Gas Chromatography*, Copyright 1966, Preston Publications, Inc.)

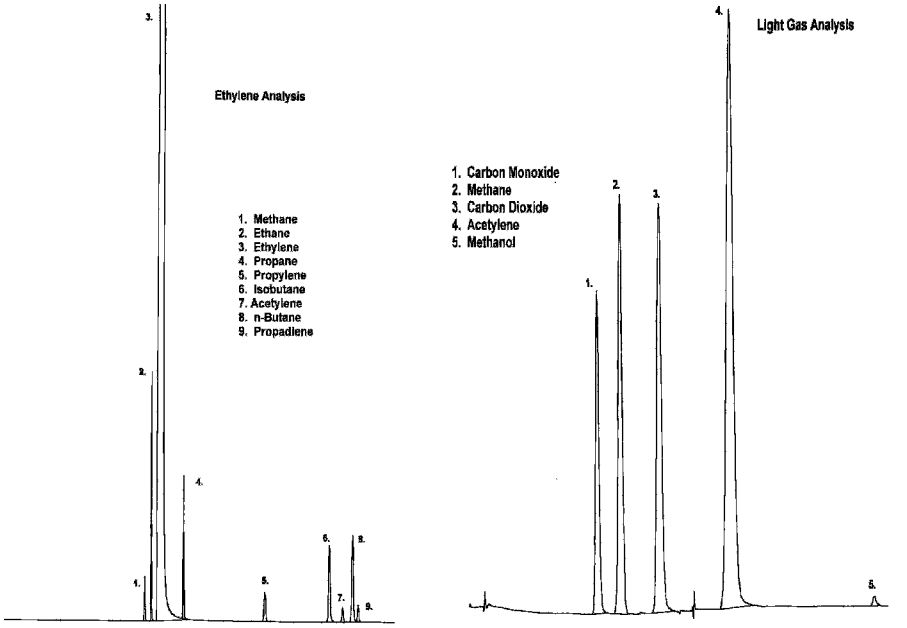


FIGURE 13.47 (a) Chromatogram of hydrocarbon impurities in ethylene. (Reprinted with permission from Reference 117, Wasson–ECE Instrumentation.) (b) Chromatogram of CO, CO_2 and methanol in ethylene. (Reprinted with permission from Reference 117, Wasson–ECE Instrumentation.)

passed through an acetylene converter that catalytically hydrogenates the acetylene. The C2 splitter then separates ethylene overhead from the ethane that is also produced. Less than 1 ppm of acetylene should be in the product ethylene. Carson et al. (118) utilized a graphitized carbon black (Carbopack B) column for these analyzes. Figure 13.48 demonstrates the effectiveness of the acetylene converter. Table 13.3 gives the concentrations for each component. Unfortunately, the hydrogen required for this conversion had to be analyzed on a 5A molecular sieve column.

13.4.1.2 Propylene

Propylene is obtained as a coproduct in the production of ethylene. A propylene splitter tower recovers the propylene from the cracker effluent after the lighter components have been removed. The determination of trace levels of ethylene, total butylenes, acetylene, methyl acetylene, propadiene, and butadiene is covered in ASTM method D2712 (19). For this analysis, 11 systems using one or two packed columns are recommended (Table 13.4). Baseline resolution is not required in this method, but a resolution requirement is given.

A total analysis for propylene impurities is very difficult. Wasson–ECE (119) has developed an automated multicolumn system to perform this analysis. One

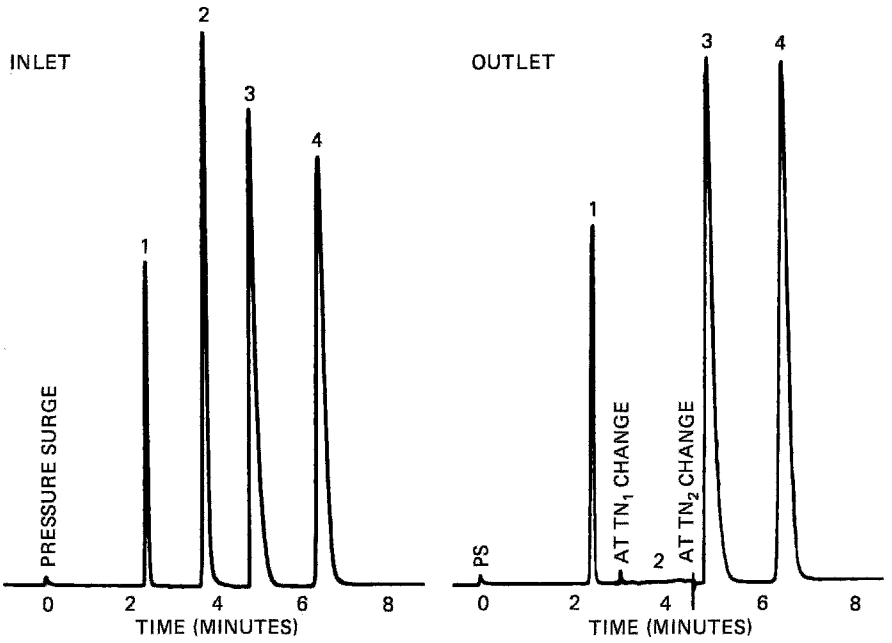


FIGURE 13.48 Chromatogram of a typical analysis of an acetylene converter inlet and outlet sample in an ethylene plant-cracking naphtha. Peak identification is contained in Table 10.3. (Reprinted with permission from Reference 118, *Journal of Chromatographic Science*, Copyright 1975, Preston Publications, A Division of Preston Industries, Inc.)

TABLE 13.3 Acetylene Converter Analyses

Peak	Component	Inlet (mol%)	Outlet (mol%)
1	Methane	0.46	0.46
2	Acetylene	1.21	>1 ppm
3	Ethylene	87.13	89.71
4	Ethane	8.64	9.83
—[a]	Hydrogen	2.55	>1 ppm

[a] Analyzed on 5A molecular sieve column and normalized into analysis.

Source: Reference 118.

subsystem uses a switching valve and capillary columns with an FID to monitor the C1–C5 paraffins and the C1–C4 olefins. The second system uses packed columns with two switching valves and an FID for the analysis of trace methane, methanol, and the individual C2 hydrocarbons. Both CO and CO_2 are determined by means of a catalytic methanizer. Typical chromatograms from the two detectors are shown in Figure 13.49.

TABLE 13.4 ASTM D2712 Column Systems for Propylene Analysis

1. 2,4-Dimethyl sulfolane (33% Chromosorb P, 0.19 in. × 4 ft); squalene (22%, Chromosorb P, 0.13 in. × 30 ft)
2. 2,4-Dimethyl sulfolane (Chromosorb P, 0.085 in. × 22 ft); β,β′-oxydipropionitrile (15% Chromosorb P, 0.085 in. × 20 ft); UCON (15%, Chromosorb, 0.085 in. × 8 ft)
3. 2,4-Dimethyl sulfolane (15%, Chromosorb P, 0.085 in. × 16 ft)
4. Silicagel (0.18 in. × 3.5 ft)
5. 1,2,3-Tris(2-cyanoethoxy)propane (20%) and SE-30 (25% Chromosorb P, 0.19 in. × 50 ft)
6. β,β′-Oxydipropionitrile (25%, Chromosorb P, 0.19 in. × 50 ft)
7. Normal hexadecane (20%, Chromosorb P, 0.085 in. × 20 ft)
8. Hexamethyl phosphoramide (30%, Chromosorb P, 0.085 in. × 20 ft)
9. Bis-2(methoxy ethoxy ethyl)ether (80%) and diisodecyl phthalate (20%, Chromosorb P, 0.085 in. × 25 ft)
10. Silicagel (modified with ferric chloride, 0.19 in. × 15 ft)
11. 2,4-Dimethyl sulfolane (33%, Chromosorb P, 0.085 in. × 8 ft); squalene (20%, Chromosorb P, 0.085 in. × 35 ft)

Source: Reference 19.

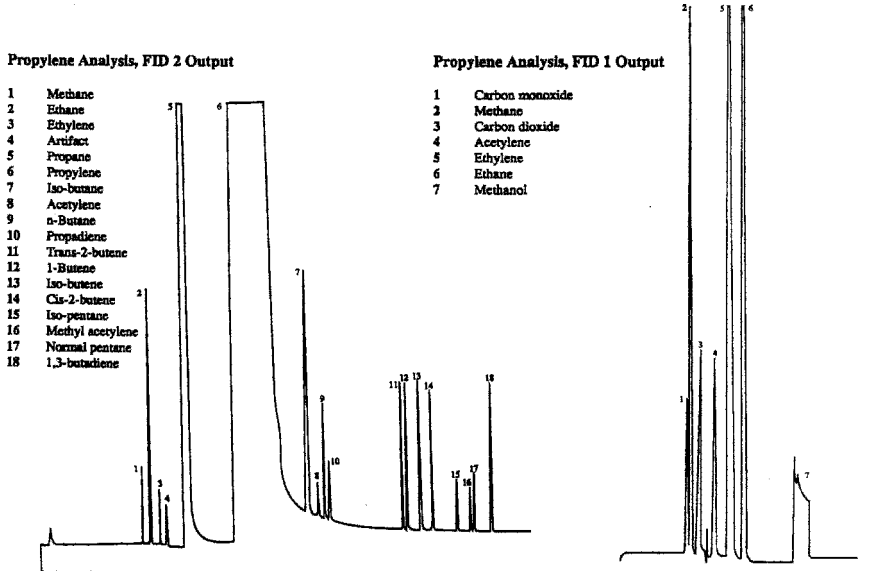


FIGURE 13.49 Chromatogram of impurities in propylene. Reprinted with permission from Reference 117, Wasson–ECE Instrumentation.)

13.4.1.3 Butadiene

High-purity 1,3-butadiene is recovered from steam cracker product streams. By using dimethyl formamide as a solvent, butadiene can be extracted from the C4 cuts.

TABLE 13.5 ASTM D2593 Cases/Column Systems for Chromatographic for Analysis of Butadiene

1. Bis-2(methoxy ethoxy ethyl) ether and diisodecyl phthalate (25%, Chromosorb P, $\frac{3}{16}$ in. × 20 ft)
2. Di-*n*-butyl maleate (15%, Chromosorb P, $\frac{3}{16}$ in. × 20 ft)
3. Bis-2-methoxyethoxy ethyl ether diisodecyl phthalate (25%, Chromosorb P, $\frac{3}{16}$ in. × 20 ft)
4. Sulfolane (30%, Chromosorb P, $\frac{1}{8}$ in. × 21 ft); didecyl phthalate (30%, Chromosorb P, $\frac{1}{8}$ in. × 3.5 ft)
5. UCON LB-550X (20%, Chromosorb P, $\frac{1}{4}$ in. × 25 ft); β,β′-oxydipropionitrile (20%, Chromosorb P, $\frac{1}{4}$ in. × 25 ft)
6. Tributyl phosphate (15%, Chromosorb P, $\frac{1}{8}$ in. × 60 ft); β,β′-oxydipropionitrile (15%, Chromosorb P, $\frac{1}{8}$ in. × 20 ft)
7. Squalene (15%, Chromosorb P, $\frac{1}{4}$ in. ×5.67 ft); dimethyl sulfolane (20%, Chromosorb P, $\frac{1}{4}$ in. × 23 ft)
8. Propylene carbonate (30%, firebrick, $\frac{3}{16}$ in. × 16 ft)

Source: Reference 19.

It can also be recovered by catalytic dehydrogenation of mixed C4 streams. Analysis of the main impurities resulting from the isolation of butadiene is covered in ASTM method D2593 (19). Eight possible columns or column combinations are suggested for this analysis (Table 13.5). Carson et al. (120) have indicated that a somewhat faster and more complete analysis can be obtained through the use of another column system. They utilized a dibutyl maleate column followed by one with bis(2-methoxyethoxy)ethyl ether as the liquid phase. As shown in Figure 13.50, all the components listed in Table 13.6 can be determined. This column was found to be useful in the analysis of butadiene plant streams for process control.

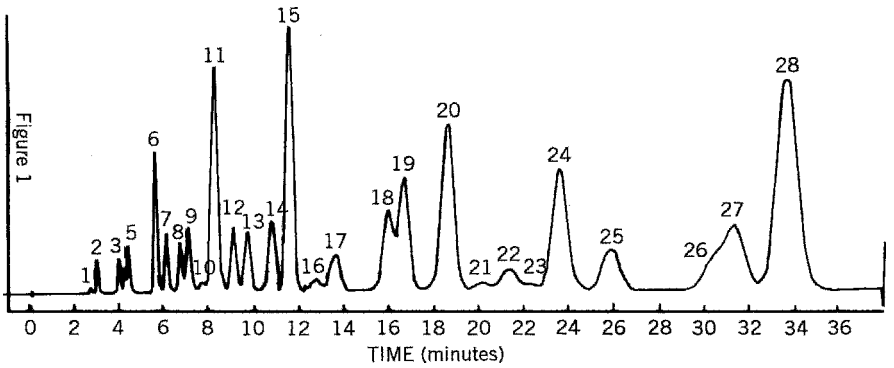


FIGURE 13.50 Analysis for butadiene process streams with peak identifications in Table 10.6. (Reprinted with permission from Reference 120, *Journal of Chromatographic Science*, Copyright 1972, Preston Publications, A Division of Preston Industries, Inc.)

TABLE 13.6 Component Identification in Butadiene Analysis

Peak Number	Component
1	Methane
2	Ethane+ethylene
3	Propane
4	Acetylene
5	Propylene
6	Isobutane
7	Cyclopropane
8	Propadiene
9	*n*-Butane
10	Neopentane
11	Butene-1-isobutylene
12	Methylacetylene
13	*trans*-Butene-2
14	*cis*-Butene-2
15	1,3-Butadiene
16	Isopentane
17	3-Methylbutene-1
18	*n*-Pentane
19	1,2-Butadiene
20	Ethylacetylene
21	2 Methylbutene-1
22	1,4-Pentadiene
23	*trans*-Pentene-2
24	Vinylacetylene
25	2 Methylbutene-2
26	2-Methylpentane
27	Isoprene
28	Dimethylacetylene

Source: Reprinted with permission from Reference 120, *Journal of Chromatographic Science*, Copyright 1972, Preston Publications, Inc.

Graphitized carbon with a light loading of picric acid has been suggested for butadiene analyses. Figure 13.51 shows a chromatogram obtained by DiCorcia and Samperi (121). This column resolves butene-1 and isobutylene, which is one of the most difficult separations in the petrochemical laboratory. Finally, the refinery gas analyzers discussed in Section 13.3.2 can also be utilized for butadiene analyses.

Since one the major uses for butadiene is the production of styrene–butadiene rubber (SBR), analyses are also required for polymerization plant recycle streams. The determination of butadiene dimer and styrene is covered in ASTM method D2426 (19). A choice of several Carbowaxes and silicone oils is given in this method.

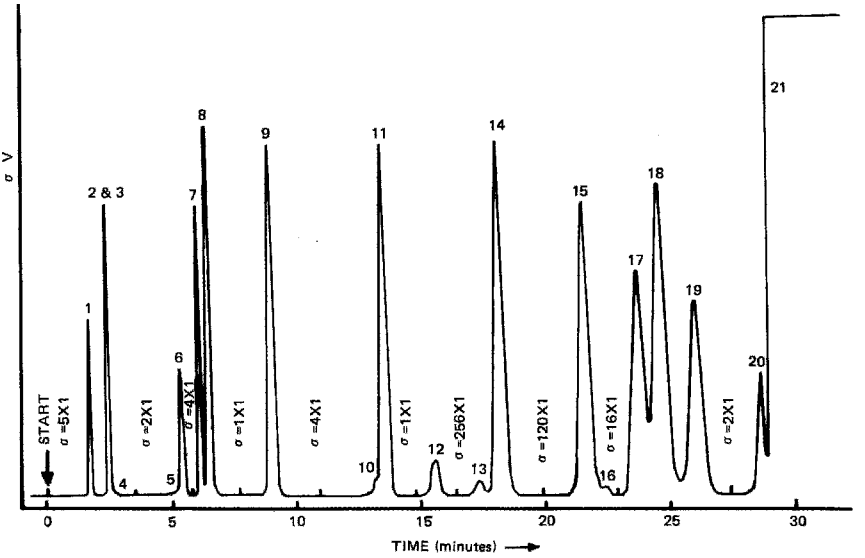


FIGURE 13.51 Analysis of trace light impurities in premium-grade 1,3-butadiene. Peak identifications are 1, methane (10 ppm); 2, ethylene (12 ppm); 3, ethane (7 ppm); 6, propane (5 ppm); 7, propene (46 ppm); 8, propadiene (70 ppm); 9, propyne (13 ppm); 11, isobutane (61 ppm); 14, 1-butene (5500 ppm); 15, isobutene (2500 ppm); 17, *cis-2*-butene (270 ppm); 18, *trans-2*-butene (390 ppm); 21, 1,3-butadiene. (Reprinted with permission from Reference 121, *Journal of Chromatography*, Copyright 1975.)

13.4.2 Aromatics

Aromatics [benzene, toluene, and xylene (BTX)] are obtained from refinery and petrochemical light naphtha streams. Aromatics are produced in the reforming process and in steam cracking. Extraction or various extractive distillation processes are used to isolate and separate aromatics from the naphtha streams. Typical extraction processes are based on tetraethylene glycol, sulfolane, N,N'-methylpyrolidene, or morpholine. They produce a mixture of aromatics that are subsequently separated by distillation, extractive distillation, or—in the case of xylene isomers—differential adsorption or fractional crystallization.

When steam cracking or naphtha reforming produce an aromatics mixture short in benzene or *o*- and *p*-xylene, some interconversion is practiced. Toluene can be hydrodealkylated to benzene. Xylene can be isomerized to increase yields of *o*- and *p*-xylene. The analysis for aromatics thus falls into two general types to meet two different needs. Analysis for process optimization assists in obtaining the maximum product at the minimum unit cost. This involves analysis of feeds, products, and raffinate (purge) streams. These analyses must be tailored to the process and the plant streams involved. Generally, it is desirable to have one analytical procedure to apply to a variety of sample types. The final product specification analysis can also be used for process control. The ASTM standard

tests for aromatic products include D2360, D2306, D3797, and D3798 (19). The latter three apply to xylene isomers.

The aromatic content of naphtha feeds to a BTX process can be measured by a procedure such as that described in ASTM method D4420 (19). Generally, a polar liquid phase is used on either an open tubular capillary or acid-washed Chromosorb P column. The aromatic content of the raffinate is used to determine the extraction efficiency and aromatic recovery of the process. A procedure similar to ASTM D4420 measures the trace levels in the raffinate (19). Stationary phases used here are also polar, and, in practice, the same chromatograph can be used for analyzing both the feed and raffinate if appropriate calibration procedures are used. The stationary phases used in ASTM D4420 are OV-275, SE-30, and OV-101.

An important process variable is the amount of extraction solvent leaving the process unit in either the raffinate or the aromatic stream. The analysis here will depend greatly on the extraction solvent used. One common feature of all of these solvents is their expense. Therefore, it is important to recover as much of the solvent as possible. Another common feature is the solvent polarity. Analysis on a polar column results in undesirably long analysis times. Analysis on a nonpolar column frequently results in a highly skewed peak and extensive tailing. For the analysis of sulfolane in BTX streams, Awwad (122) used a mixed stationary phase of 2% Carbowax 20 M in SE-30. The resulting analysis is shown in Figure 13.52.

Analysis of product benzene and toluene is covered by ASTM D2360. The method suggests Carbowax 1540 (25%) on Chromosorb P (60/80 mesh). Any stationary phase yielding the specified resolution can be used. An internal standard (*n*-butyl benzene) is used.

Analysis of mixed xylenes is by ASTM D2306 with the use of a crosslinked poly(ethylene glycol) (e.g. Carbowax 20 M) stationary phase. Both capillary and packed columns are acceptable. Figure 13.53 illustrates this separation on a SP-1200/Bentone 34 column (123).

Analysis of product xylenes is by ASTM D3797 (*o*-xylene) or D3798 (*p*-xylene). These procedures are similar, and satisfactory columns are given in Table 13.7. The concentration of impurities is measured by internal standard calibration and the purity of the *o*- or *p*-xylene is determined by subtracting the percent impurities from 100%.

13.5 PROCESS CHROMATOGRAPHY

Process GC has developed into one of the most widely used online monitoring techniques in the petrochemical industry. Its popularity is due to the ability of GC to quickly analyze hydrocarbon streams for process control. As is the case for all on-line analyzers, process chromatographs are capable of safe, continuous, unattended, in-plant operation. Because of the need for fast and specific analyses to provide feedback for process control, these chromatographs are usually designed for each specific application. The ideal process gas chromatograph (PGC) has the following characteristics:

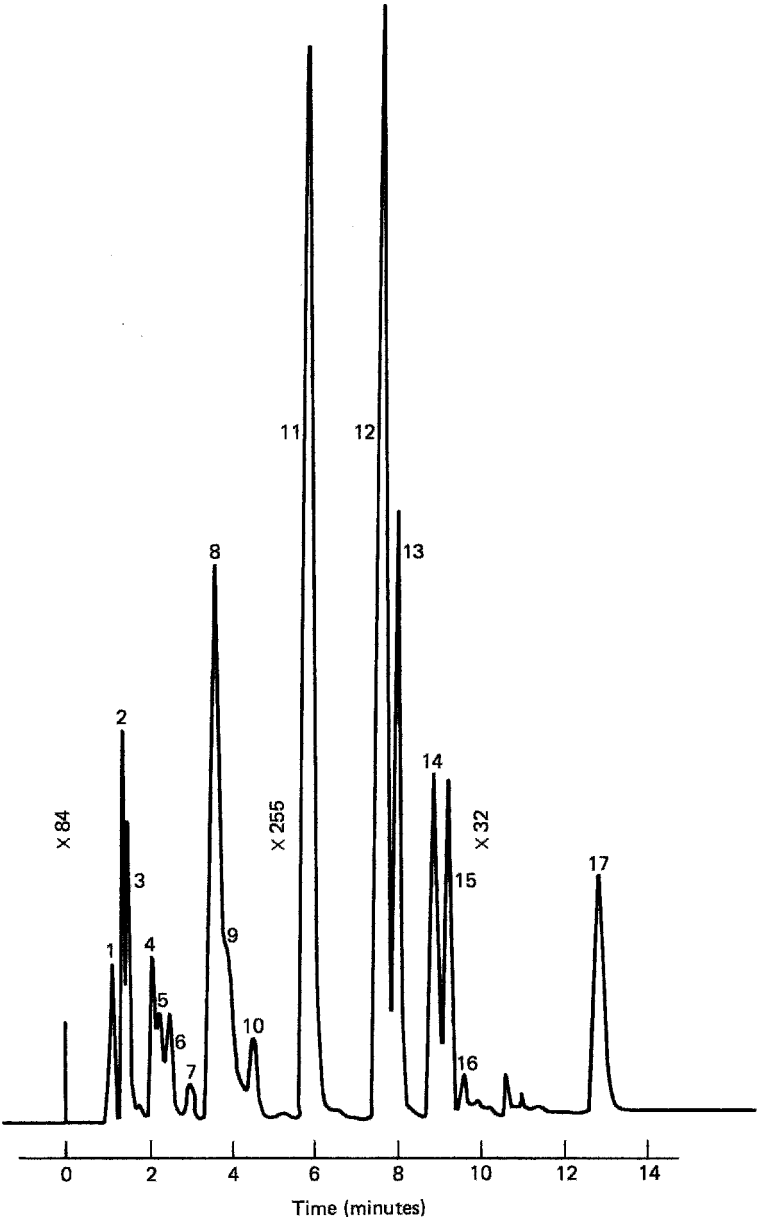


FIGURE 13.52 Typical chromatogram of sulfolane in BTX mixture extracted from Iragi Powerformate. Peak identifications are 1, *n*-pentane; 2, *cis*-2-pentene; 3, 2-methyl-2-butene; 7, *n*-hexane; 8, benzene; 9, cyclohexane; 10, 2,4-dimethylpentane; 11, toluene; 12, *m*- and *p*-xylene; 13, *o*-xylene; 14, *n*-propylbenzene; 15, *tert*-butylbenzene; 16, isobutylbenzene; 17, sulfolane. (Reprinted with permission from Reference 122, *Journal of Chromatographic Science*, Copyright 1979, Preston Publications, A Division of Preston Industries, Inc.)

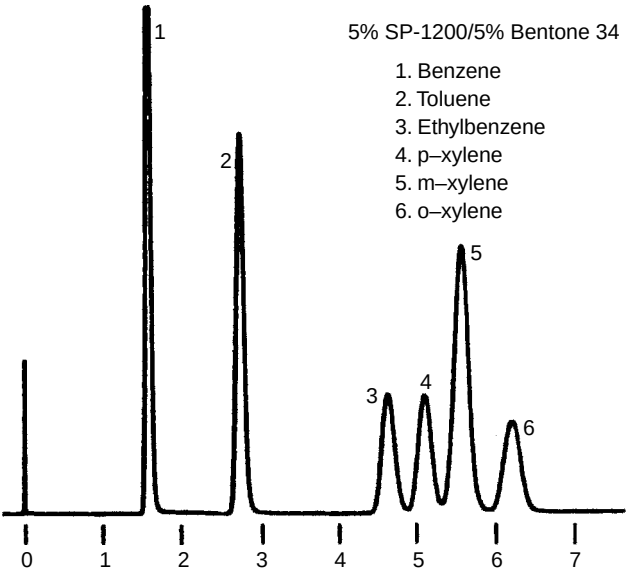


FIGURE 13.53 Chromatogram demonstrating the separation of aromatics on a 5% SP-1200/5% Bentone 34 on 100/120-mesh Supelcoport (6 ft × $\frac{1}{8}$ in.) at 100°C (82). (Reprinted from Reference 123 with permission of Supelco, Inc, Bellefonte, PA 16823.)

TABLE 13.7 Typical Columns for Analysis of Impurities of Xylenes

ASTM D-3797 o-Xylene
1. Butylbenzyl tetrachlorophthalate (SS 200 ft × 0.01 in.)
2. Dibutyl tetrachlorophthalate (SS 250 ft × 0.02 in.)
3. Di-*n*-propyl tetrachlorophthalate (SS 200 ft × 0.02 in.)
4. Carbowax 1540 (SS 300 ft × 0.01 in.)
5. Bentone 34 (5%)/OS 124 (5%) (Chromosorb WAW, SS 10 ft × $\frac{1}{8}$ in.); 1,2,3-Tris(2-cyanoethoxy) propane (20%) (Chromosorb PAW, SS 18 ft × $\frac{1}{8}$ in.)
ASTM D3798 p-Xylene
1. Polyethylene glycol or Carbowax 20 M (fused silica, 50 m × 0.32 mm)
2. Diisodecylphthalate (3.5%) or Bentone 34 (Chromosorb W, 6.1 m × 3.2 mm)

Source: Reference 19.

- Simple, reliable design to ensure a low failure rate
- Design that provides for simple, rapid repair
- Sensors strategically placed in the system to aid in diagnosis of problems
- Alarms to monitor proper operation

The main components of the process gas chromatograph include a sample system, the gas chromatographic analyzer, and a programmer. A schematic of this

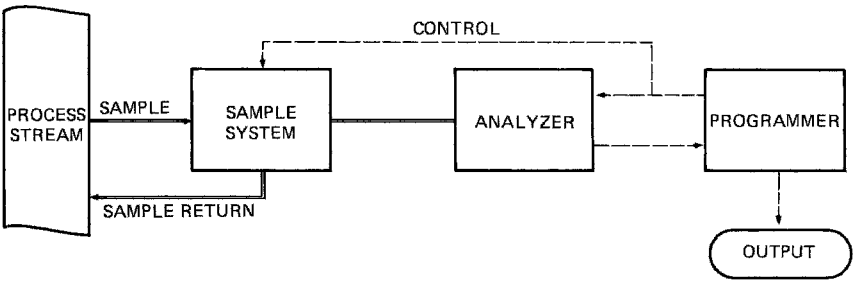


FIGURE 13.54 Simplified schematic of a process gas chromatographic analyzer system.

system is shown in Figure 13.54. The sample system continuously circulates the process stream or streams of interest in the analyzer. Sample systems are designed to avoid the problems encountered in sampling for laboratory analyses. The analyzer is basically a gas chromatograph, but is designed for explosion proof operation in the plant environment. The required separations are usually achieved with the use of valve switching and multiple columns. The programmer is a computer that handles the operation of the chromatograph along with several sample streams. It also handles the data as well as any required computations, such as calorific [British thermal unit (BTU)] content. Currently, the chromatograph and programmer are integrated into a standalone unit. It can also be interfaced with other process control systems.

Overall, the trend is toward increased usage of process gas chromatographs in the future. This is based on the need for plant optimization as well as increased reliability and reduced maintenance of these on-line analyzer systems. Several reviews of this type of analyzer and their manufacturers have been recently published (124–131).

13.5.1 Process Chromatographs

13.5.1.1 Sample System

The sample system is the most critical part of the process analyzer. It is designed to provide a constant flow of sample to the analyzer. This includes conditioning of the sample stream so that a representative sample can be injected into the chromatograph. Thus the composition of each process stream must be carefully considered so that the sample system can be designed appropriately. Complete details of these considerations are discussed by Sherman (132) and in the earlier work by Houser (133). Unfortunately, the majority of the failures of process chromatographs can be attributed to the sample-handling system.

The sample stream originates from the process through a sample probe. The probe is inserted through a process valve into the center of the process stream. This provides a representative sample by minimizing the effects of laminar flow, gas bubbles, condensed liquids, and particulates.

The sample line itself is designed to provide a representative sample to the analyzer within the cycle time of the analysis. Typically, sample flow is obtained

by the pressure drop between properly selected sample and return points. If necessary, a pump may be utilized, but this is expensive. The size of the sample line is a function of the required flow, the available pressure drop, and the length of the line. Relatively short and straight lines are the most desirable. Stainless-steel tubing is the most common choice for sample lines. The reactivity of the process stream, however, must be considered in choosing the proper materials. For example, the presence of hydrochloric acid requires the use of Monel. Insulation and heat tracing of the line may also be required to maintain the composition of the stream as well as to control the flow of viscous fluids. For gas streams, the possibility of condensation must be eliminated. The temperature and pressure of a liquid stream must be maintained to prevent condensed gases from reaching their bubble point. Tubing is now available that is manufactured with insulation and either steam or electrical tracing.

Most sample streams require filtration to remove particulates. Phase separation may also be required for removal of condensed liquid droplets from vapors or immiscible droplets from liquids. These are usually accomplished by slipstreaming the sample through a filter, as shown in Figure 13.55. Only periodic flow to the sample valve actually passes through the filter element. This arrangement greatly reduces the maintenance on the system. From the filter, automatic valve switching is used to provide flow to the sample valve for injection. Valve switching is also used to sample multiple streams with a single analyzer. Flow controllers are installed on each line.

A vaporizer is utilized to convert mixed-phase samples into gases prior to sampling. All of these components are contained in a temperature-controlled, air-purged cabinet. Valving and a vaporizer, if necessary, are provided for analysis of a calibration standard. Further details on calibration of the analyzer are discussed below.

13.5.1.2 Analyzer

The analyzer automatically performs the functions of a typical laboratory gas chromatograph. The key to the success of the process analyzer is the reliability of the sample valve. Multiport valves are used for sampling liquids or gases as well as column switching and backflushing. The most widely used valves include the diaphragm, sliding plate, O-ring, and rotary valves. A liquid injection valve with a built-in vaporizer is also available. A sample loop is usually used to control the sample volume of gases. An internal hole or channel is used for liquids with pressures of up to 200 psig. All of these valves can be obtained with special metals or coatings for reactive or corrosive streams.

The columns in the analyzer are arranged to provide repetitive analyses within the minimum amount of time possible. Analyses typically are performed within several minutes. To accomplish this, multiple columns with valve switching are used. The porous polymer column packings are widely used because of their high efficiency and stability. The term “stability” implies low bleed and resistance to normal and upset condition components in the sample. With the development

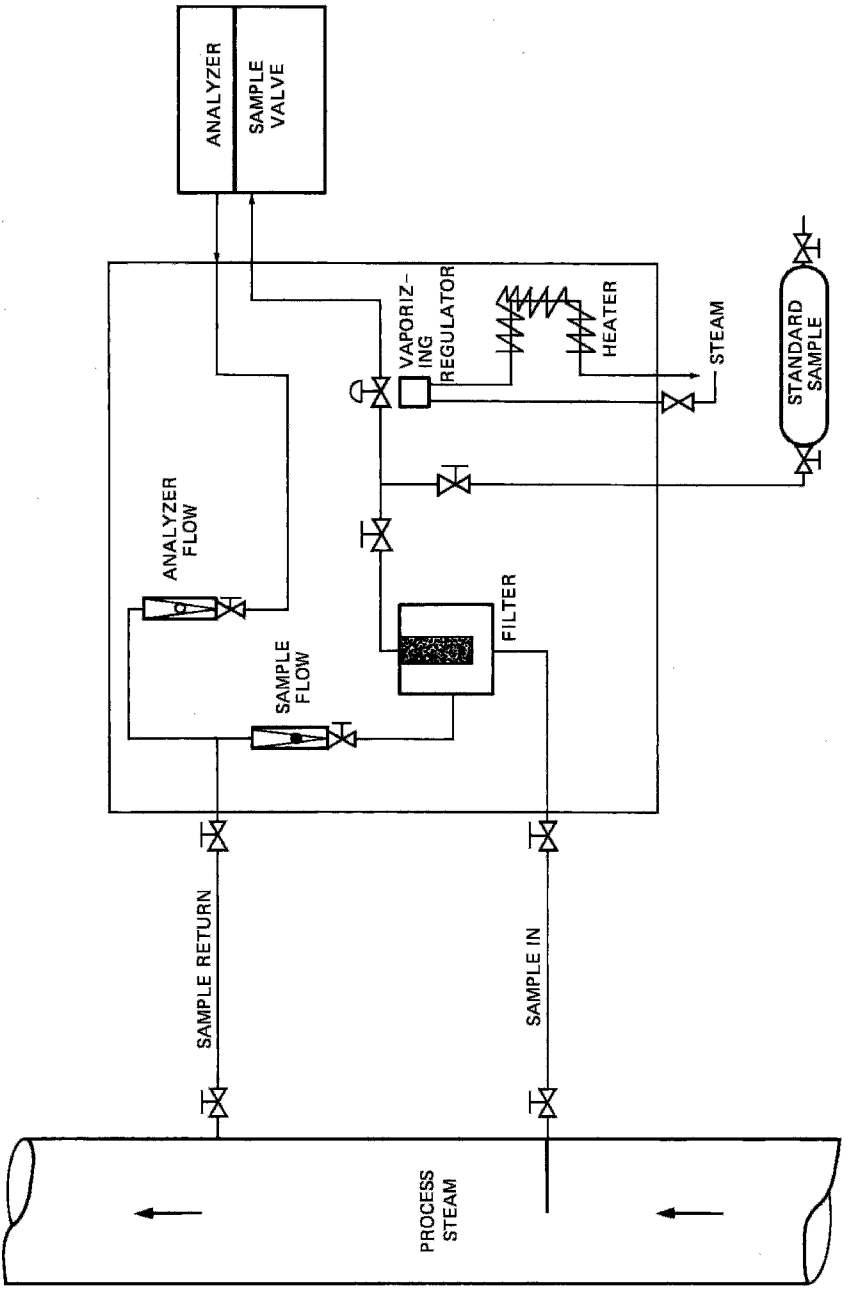


FIGURE 13.55 Typical schematic of a sampling system for a process gas chromatographic analyzer system.

of fused-silica columns, capillary columns are now being utilized. Most analyses are performed isothermally, although temperature-programmed analyzers are available.

Multicolumn techniques provide fast analyses by separating and purging the nonquantified components. The most widely used technique is backflushing. The backflush-to-measure method involves analysis of the initial components through the columns. Flow is then reversed through the column, and the remaining components are measured as a single peak. The backflush-to-vent method uses two columns. The precolumn is used to separate the components of interest from the unwanted components. The components of interest pass on to the analysis column where they are further separated. While this is occurring, the unwanted components are backflushed from the precolumn and vented. Another technique utilizes dual-analysis columns. Resolved components from the first column are sent to the detector while unresolved components are passed to the second column for further separation. The final technique is heartcutting. It is used mainly to measure a trace component that is not fully resolved from a major component. The method uses two columns, with the first column performing the primary separation. A narrow cut from the first column, which contains the component of interest along with a portion of the major component, is transferred to the second column for further separation. Each of these techniques is shown schematically in Figure 13.56.

The thermal conductivity detector and the FID are used most frequently. For hazardous areas, a pneumatic composition transmitter is used. It monitors the changes in differential pressure across an orifice for detection of the components as they elute (134). The FPD is available for determination of low levels of sulfur or phosphorous compounds. Oxygenated or chlorinated compounds can be monitored by use of an electron-capture detector (ECD).

For these concentration-type detectors, control of the carrier-gas flow is critical to maintain repeatable peak areas and retention times (136). Pressure regulation is typically used as it responds quickly to valve switching as well as compensating for leaks. Electronic controllers offer the additional option of pressure to reduce analysis cycle time.

13.5.1.3 Programmer

The *programmer*, also referred to as the *controller*, operates the sample system and the analyzer. In addition to this, it handles collection and presentation of the chromatographic data. The controller is now a computer that has replaced the microprocessor as well as the original cam timers. Its basic function is to control valve switching for multiple streams and calibration standards in the sample system. For the analyzer, it controls the operation of the sample inject and column switching valves. Multiple streams can be handled with different valve switching times and sequences. The detector can also be automatically zeroed.

In addition to timing, the programmer handles collection and reduction of the chromatographic data. Since only a few specific components are analyzed, the technique of gating is used. Gating integrates only the very small time band

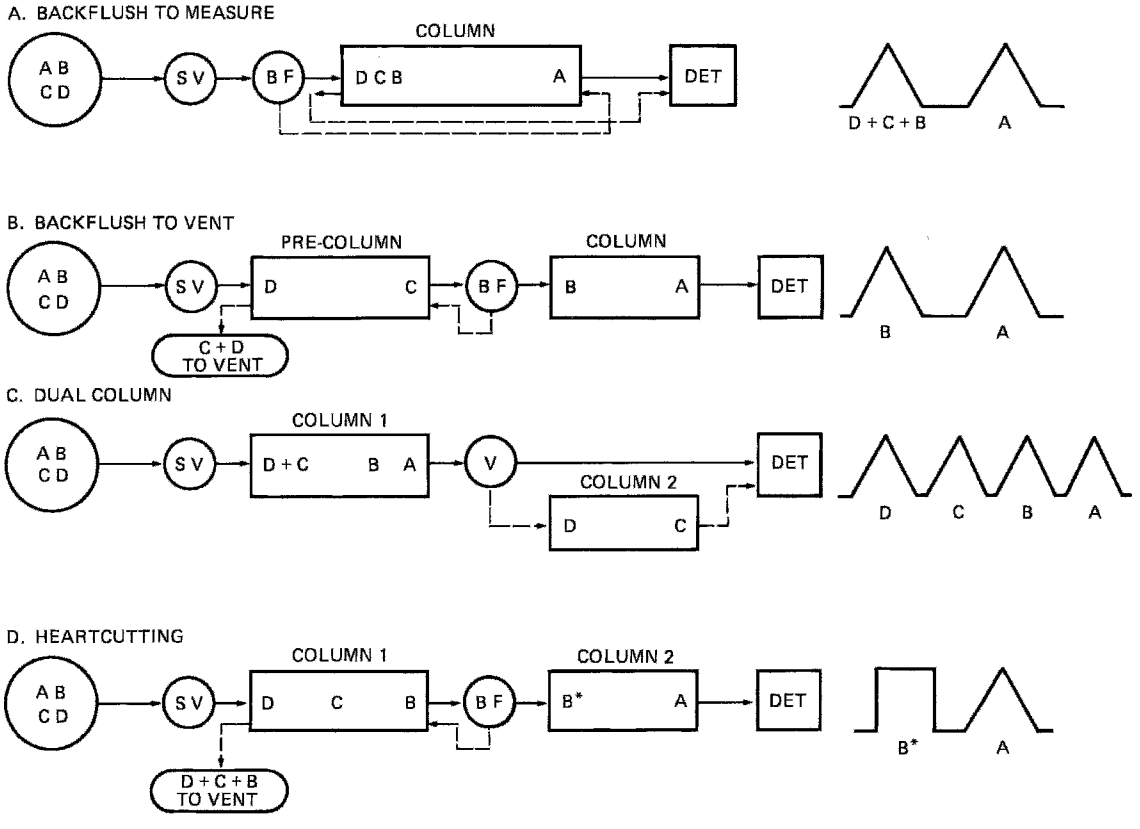


FIGURE 13.56 Simplified schematics and chromatograms for the multicolumn techniques used in process gas chromatographs (SV, sample valve; BF, blackflush valve). Dashed lines indicated flow pattern during backflush.

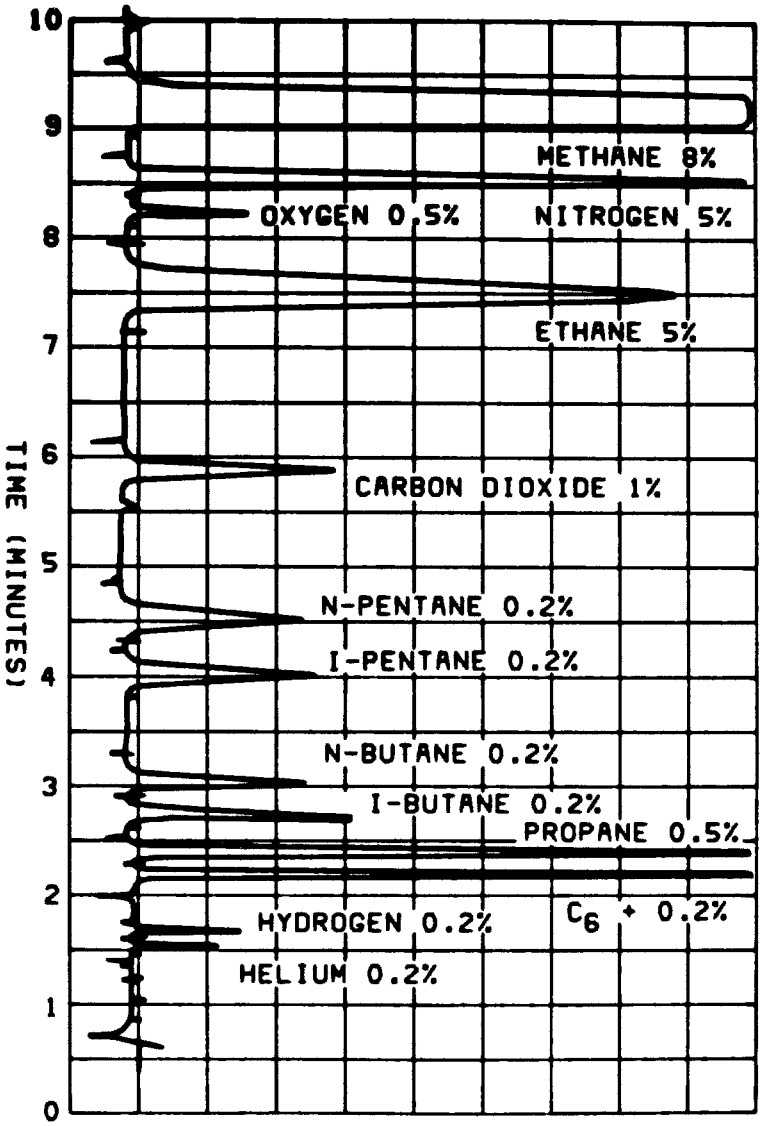
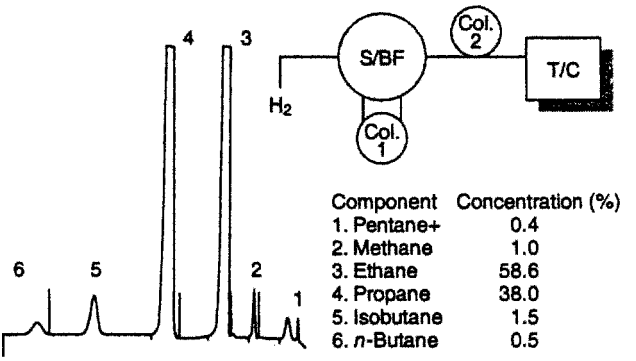


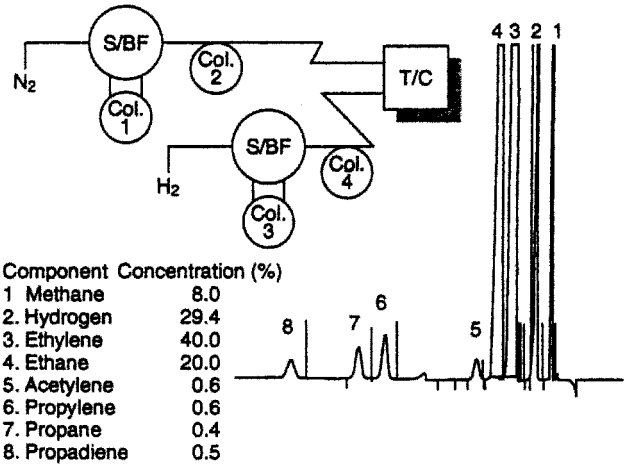
FIGURE 13.57 Typical chromatogram from a BTU content analyzer for natural gas streams. (Courtesy of Applied Automation/Siemens, Bartlesville, OK 74005.)

in which the component elutes. Quantification is provided by comparison to external standards. The newer systems can use an initial slope to detect the start of a peak. Tangent skimming and dropping of a perpendicular for unresolved peaks is also possible.

As mentioned earlier, quantification is usually achieved by comparison to external standards. However, internal normalization and comparison to laboratory



Ethylene furnace feed chromatogram.



Furnace effluent chromatogram.

FIGURE 13.58 Typical process gas chromatograms and column configurations for a gas cracking furnace inlet (a) and outlet (b). (Courtesy of ABB Automation, Inc., Lewisburg, WV 24901.)

analyses can also be used. External standards are blends of known composition or a process stream that has been analyzed. The process stream analyses are then correlated with analysis of the calibration standard. An important consideration for these standards is that they must be stable over long periods of time. Care must be taken with components that may react with other components or the walls of the container. Loss of a very volatile component into the vapor space of a liquid standard must also be considered.

The programmer is typically networked with a process control computer. Both system and data alarms are also transmitted. Another pathway is provided to direct maintenance data to an analyzer management station. This allows for statistical

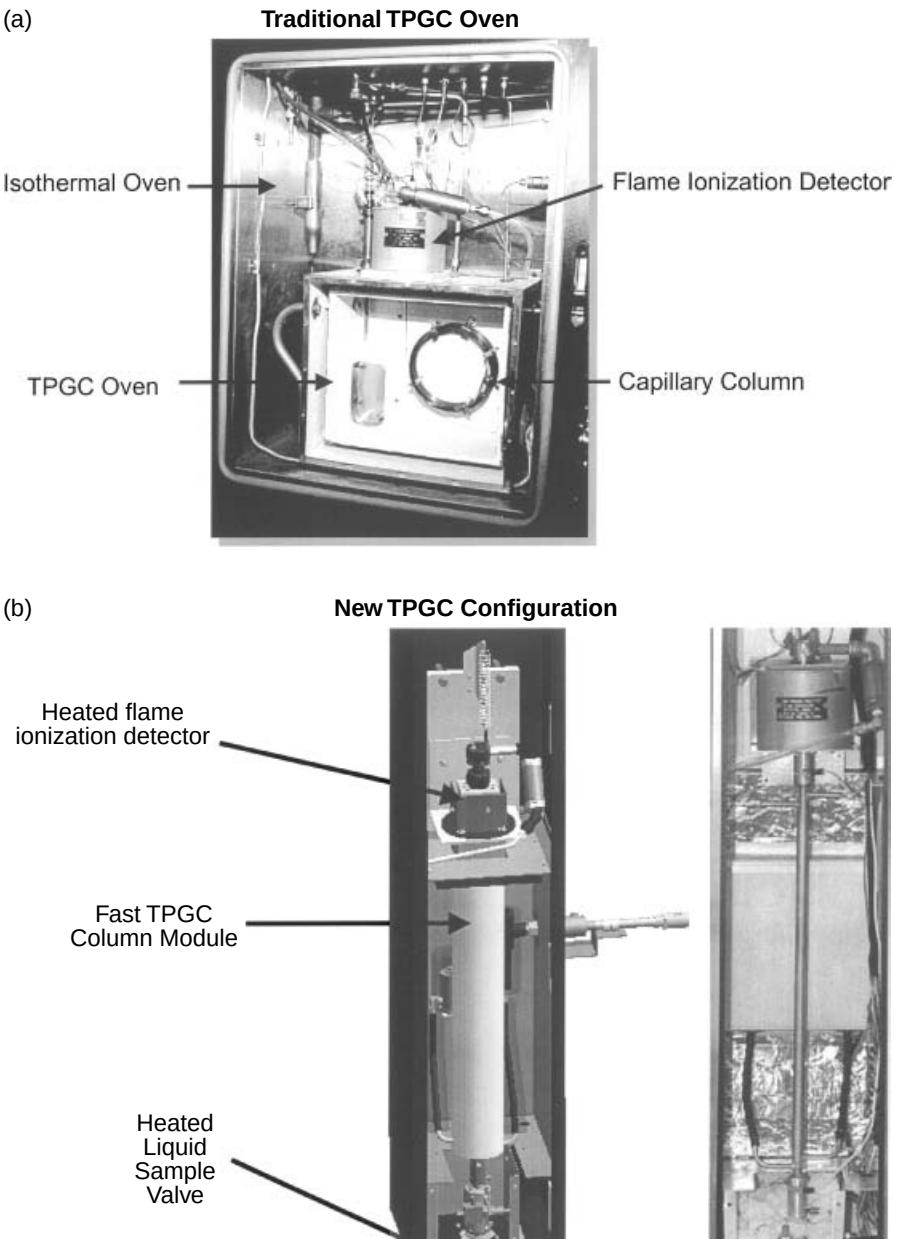


FIGURE 13.59 Comparison of a traditional (a) and fast (b) process temperature programmed gas chromatograph configuration. (Courtesy of ABB Automation, Inc., Lewisburg, WV 24901.)

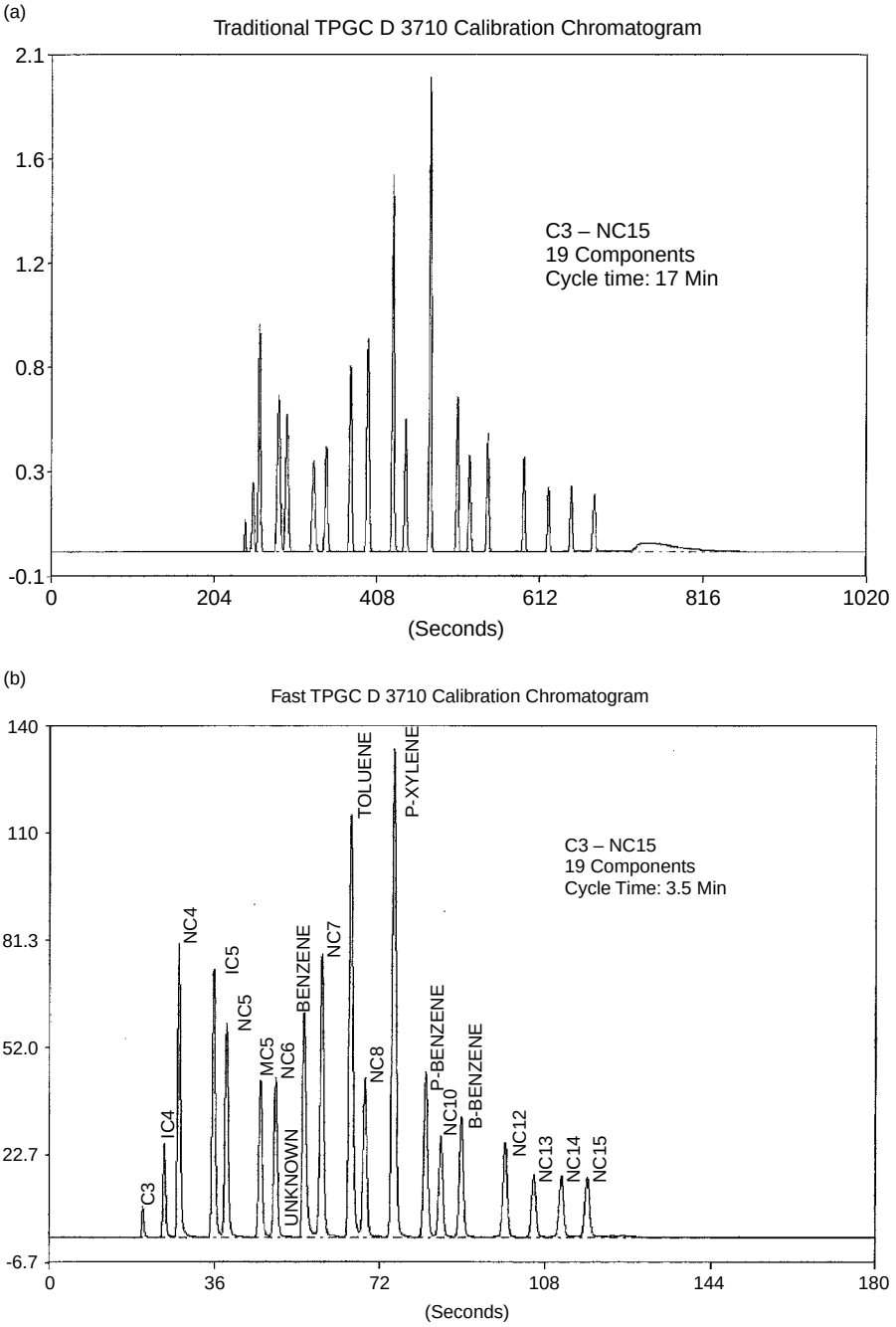


FIGURE 13.60 Comparison of a traditional (a) and fast (b) process gas chromatogram for an ASTM D3710 standard as well as a gasoline sample. (Reprinted from Reference 136 with permission from ABB Automation, Inc., Lewisburg, WV 24901.)

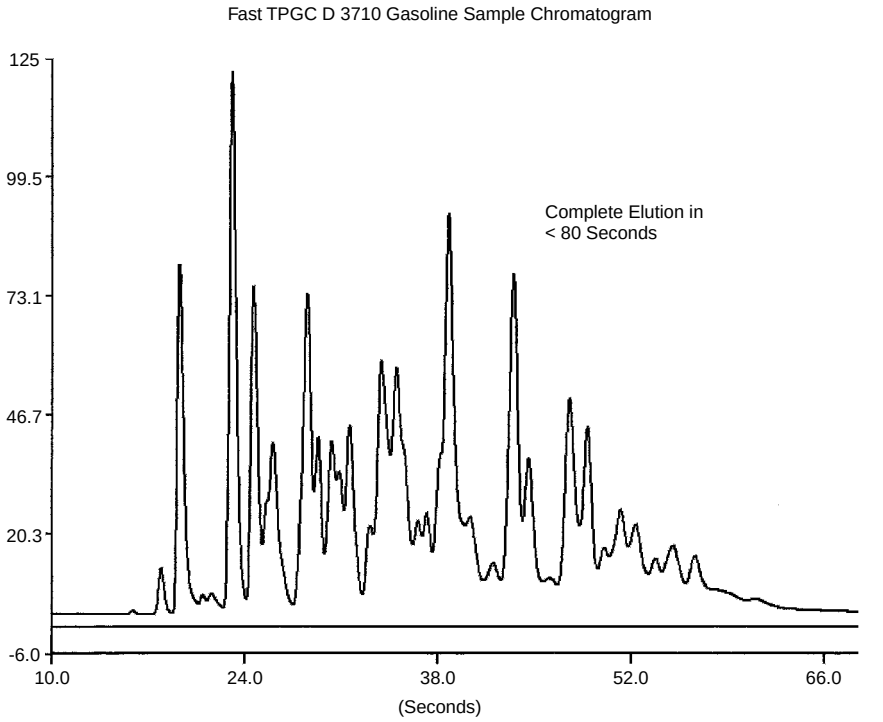


FIGURE 13.60 (*Continued*)

monitoring of the performance of the analyzer. Reprogramming can be done either through this station or at the analyzer itself.

13.5.2 Typical Applications

The most predominant application of the process gas chromatograph is to provide feedback for process control, in either open- or closed-loop control. In the case of a fractionation tower, one or more components are monitored, and the result then directly controls the operation of the tower. Griffen et al. (135) summarized the considerations involved in analyzer control of fractionators. More complex analyses are required for applications such as cracking products, polymerization reactor feed impurities, and high-purity product quality control. Systems for determination of boiling point distributions and octane ratings of refinery streams have also been developed.

Figure 13.57 is a typical chromatogram from an analyzer for BTU content of natural gas streams. In this analysis, the individual components are analyzed and then the BTU content and specific gravity are calculated. In order to control a gas cracking furnace to produce ethylene, the inlet and outlet are monitored with a multicolumn system as shown in Figure 13.58. The speed and resolution

achieved in these examples through column switching techniques should be noted. It is possible to obtain typical analyses in less than 30 s through the use of microcolumns. Figure 13.59 compares a typical process gas chromatograph with a new design that allows for faster temperature programming and cooldown (136). A comparison of calibrations for traditional and fast process gas chromatographs is shown in Figure 13.60 along with an analysis of a gasoline sample within 80 s.

These analyses demonstrate the potential of the process chromatograph. Undoubtedly, they will continue to be used to improve the efficiency of refinery and chemical plant operations.

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This chapter is also dedicated to the memory of the original co-author, Kenneth E. Paulsen, particularly for his contributions to the section on exploration and production.

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CHAPTER FOURTEEN

Clinical and Pharmaceutical Applications of Gas Chromatography

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14.1 INTRODUCTION

In this chapter, the current clinical applications of gas chromatography (GC) are described. Rather than providing a compilation of all existing methodologies for the gas chromatographic analysis of the various drugs herein described, the author has included those analytical procedures that are more frequently used in clinical laboratories and that provide high resolution, accuracy, and high speed of analysis. The pharmacological properties of the drugs, including pharmacological effects, mechanism of action, and absorption and elimination, and a detailed description of the procedures used for their respective chromatographic analysis are included in each section. In this way, the reader will have an opportunity to become familiar with the chemical properties and the pharmacodynamics and pharmacokinetics of each class of drugs before a detailed description of the methods of analysis is presented.

14.2 AMPHETAMINES

Amphetamines comprise a group of adrenergic agonists that include amphetamine, methamphetamine, methylphenidate, pemoline, ephedrine, ethylnorepinephrine, phenmetrazine, benzphetamine, phendimetrazine, phenmetrazine, diethylpropion, mazindol, fenfluramine, and phenylpropanolamine. Under federal regulation, amphetamines are considered schedule II drugs. Classical therapeutic applications of amphetamines include the treatment of obesity and narcolepsy. These drugs were found to produce weight loss by suppressing appetite (anorexia) rather than by increasing energy expenditure. Adverse effects of treatment include the potential for drug abuse and habituation, serious worsening of hypertension, sleep disturbances, palpitations, dry mouth, and depression.

In this section, the generic concept of sympathomimetic drugs and their pharmacological behavior is introduced, followed by GCMS analysis of amphetamines.

14.2.1 Pharmacological Considerations

14.2.1.1 Chemistry and Structure

ß-Phenylethylamine can be considered as the parent compound of sympathomimetic amines, consisting of a benzene ring and an ethylamine sidechain.

The structure permits substitutions to be made on the aromatic ring, the α- and β-carbon atoms, and the terminal amino group, to yield a great variety of compounds with sympathomimetic activity. Norepinephrine, epinephrine, dopamine, isopropterenol and a few other compounds have OH groups substituted at the 3 and 4 positions of the benzene ring. Since *O*-dihydroxybenzene is also known as catechol, sympathomimetic amines with OH substitutions in the aromatic ring are designated catecholamines. Since substitution of polar groups on the phenylethylamine structure makes the resultant compound less lipophilic, alkyl-substituted compounds, including the amphetamines, cross the blood–brain barrier more readily and have more activity in the central nervous system.

14.2.1.2 Pharmacological Effects

The sympathetic nervous system is involved in the homeostatic regulation of a wide variety of functions, including heart rate, force of cardiac contraction, vasomotor tone, blood pressure, bronchial airway tone, and carbohydrate and fatty-acid metabolism. Stimulation of the sympathetic nervous system normally occurs in response to physical activity, psychological stress, and generalized allergic reactions. The host of physiological and metabolic responses that follow stimulation of sympathetic nerves in mammals is usually mediated by the neurotransmitter norepinephrine. As part of the response to stress, the adrenal medulla is also stimulated, resulting in elevation of the concentrations of epinephrine and norepinephrine in the circulation. The actions of these two catecholamines are very similar at some sites but differ significantly at others. For example, both compounds stimulate the myocardium; however, epinephrine dilates blood vessels in skeletal muscle, whereas norepinephrine has a constricting effect. Dopamine is a third naturally occurring catecholamine. Although it is found predominantly in the basal ganglia of the central nervous system (CNS), dopaminergic nerve endings and specific receptors for this catecholamine have been identified elsewhere in the CNS and in the periphery. As might be expected, naturally occurring sympathomimetic amines and drugs that mimic their actions, comprise one of the more extensively studied groups of pharmacological agents. Some of the functions ascribed to catecholamines are summarized in Table 14.1.

14.2.1.3 Mechanism of Action

Crucial to understanding the remarkably diverse effects of the catecholamines and related sympathomimetic agents is an understanding of the classification and properties of the different adrenergic receptors. Although structurally related, different adrenergic receptors regulate distinct physiological processes by controlling the synthesis of a variety of second messengers. Ahlquist in 1948 first proposed that there was more than one adrenergic receptor (1). He proposed the designations α and β for receptors in smooth muscle where catecholamines produce excitatory and inhibitory responses, respectively. Stimulation of α1 receptors results in activation of phospholipase C through mediated by a G protein, and the hydrolysis of membrane-bound polyphosphoinositides with the generation of the second messengers, diacylglycerol and inositol-1,4,5-triphosphate which

TABLE 14.1 Pharmacological Actions of Sympathomimetic Drugs

Peripheral excitatory	*Contraction* of smooth muscle in vessels supplying skin and mucuos membranes; gland cells
Peripheral inhibitory	*Relaxation* of smooth muscle in the wall of the gastrointestinal tract; bronchial tree; and in blood vessel supplying the skeletal muscle.
Cardiac excitatory	*Increase* in the heart rate and force of contraction.
Metabolism	*Increase* in rate of glycogenolysis in liver and muscle; and liberation of fatty acids from adipose tissue.
Endocrine system	*Modulation* of secretion of insulin, renin, and pituitary hormones.
CNS	*Stimulation* of respiration and increase in wakefulness, psychomotor activity, and a reduction of appetite.

stimulate the release of Ca^{2+} from intracellular stores (2). A major component of the responses that follow involves regulation of several protein kinases, including protein kinase C. For example, α1 adrenergic receptors regulate hepatic glycogenolysis. This results from the activation of phosphorylase kinase by the mobilized Ca^{2+} and is aided by the inhibition of glycogen synthase caused by protein kinase C-mediated phosphorylation (2).

Sympathomimetic drugs influence both α and β receptors in the target tissue, but the ratio of the α and β activity varies widely between drugs, in a continuous spectrum from an almost pure α activity (phenylephrine) to an almost pure β activity (isopropterenol). An important factor in the response of any cell or organ to sympathomimetic amines is its density and proportion of α and β receptors. For example, norepinephrine has relatively little capacity to increase bronchial airflow since the receptors in bronchial smooth muscle are largely of the β_2 type. In contrast isopropterenol and epinephrine are potent bronchiodilators. Cutaneous blood vessels possess α receptors almost exclusively; thus epinephrine and norepinephrine cause marked constriction.

14.2.1.4 Absorption and Elimination

Epinephrine and norepinephrine are not effective by oral administration because of their rapid conjugation and oxidation in the gastrointestinal mucosa and liver. Absorption from subcutaneous tissues occurs slowly because of local vasoconstriction. Absorption is more rapid after intramuscular than after subcutaneous injection. Epinephrine is rapidly inactivated in the body. The liver, which is rich in both of the enzymes responsible for the metabolization of circulating epinephrine (catecholamine methyl transferase and monoamino oxidase), is particularly important in this regard. Isopropterenol is also given parenterally or as an aerosol and it is rapidly metabolized in the liver but unlike epinephrine and norepinephrine is not inactivated by enzyme monoamino oxidase. The selective α_2 adrenergic agonists, clonidine, guanfacine, guanabenz, and methyldopa normally used in the treatment of hypertension, are well absorbed orally and have a

large volume of distribution and can be administered as tablets. Amphetamines in the form of sulfate salts are well absorbed orally.

14.2.2 Gas Chromatographic Analysis

14.2.2.1 Sample Preparation

The procedure described in this section is a modification of that described by Namera et al. (3), where ethylchloroformate is used for amphetamine derivatization in order to achieve high resolution of the various amphetamines. Blood or urine samples (0.5 mL),1 mL of K_2CO_3, 0.5 g of NaCl, 20 μL of ethylchloroformate, and 30 μL of the internal standard, are placed into a 10-mL vial and sealed rapidly with a silicon septum and a vial cap. The SPME needle is inserted into the vial and extraction fiber exposed in the headspace. The vial is then heated at 80°C for 15 min. The vial is rotated at 250 rpm during the SPME extraction. After extraction, the fiber is pulled back into the needle and the needle inserted into the injection port of the GCMS instrument. The fiber is exposed for 3 min in the injector.

14.2.2.2 Analytical Procedure

A Shimadzu GC-MS instrument, model GC-17A/QP-5000 equipped with a 30 × 0.25-m-i.d. fused-silica capillary column (Supelco, PTE-5, 0.25 μm film thickness) is used for analysis. The oven temperature was set at 80°C for 3 min and then programmed from 80 to 220°C at 40°C/min, from 220 to 280°C at 8°C/min, and held at 280°C for 3 min. The temperature of the injection port and the interface were set at 250 and 230°C, respectively. The splitless injection mode was used. Helium at a flow rate of 0.8 mL/min was used as a carrier gas. The ions used for quality were m/z 91, 102, 130, and 221 for methamphetamine; m/z 91 and 130 for phentermine; m/z 72, 116, 144, and 159 for fenfluoramine; m/z 91, 102, and 193 for phenylethylamine; m/z 102, 128, 230, 242, and 332 for 2C-B; m/z 116, 135, and 251 for methylenedioxyamphetamine (MDA); and m/z 102, 130, 135, and 265 for methylenedioxymethamphetamine (MDMA) at selected ion monitoring. Ions used for quantitation were m/z 102 for phenylethylamine; m/z 116 for amphetamine and MDA; m/z 130 for methamphetamine, MDMA, and phentermine; m/z 144 for fenfluoramine; m/z 230 for 2C-B and m/z 134 for methamphetamine-d_5 (internal standard).

14.2.2.3 Quantification of Amphetamines

The method described herein allows the analysis of the eight most commonly used amphetamines. The order of elution was phenyletylamine, followed by amphetamine, phentermine, fenfluoramine, methamphetamine, MDA, MDMA, and 2C-B. The limit of detection, linearity range, and correlation coefficients of the calibration curves obtained for the different amphetamines are shown in Table 14.2. The intraday and interday coefficients of variation for concentrations of 5 ng/mL, 50 ng/mL, and 500 ng/mL were 1.17%, 2.98% and 12.2%, respectively.

TABLE 14.2 Quantification Limit and Linearity for the Analysis of Amphetamines

Compounds	Limit of Detection (ng/mL)	Range of Linearity (ng/mL)	*r* Value
Amphetamine	0.5	2.0–1000	0.999
Methamphetamine	0.5	1.0–1000	0.999
Phentermine	0.5	2.0–1000	0.999
Fenfluoramine	0.5	2.0–1000	0.999
Phenylethylamine	5.0	10–1000	0.999
MDA	2.0	5.0–1000	0.996
MDMA	0.5	1.0–1000	0.998
2C-B	10	50–1000	0.995

14.3 INHALATIONAL ANESTHETICS

14.3.1 Pharmacological Considerations

14.3.1.1 Chemistry and Structure

Halothane (Fluothane) is 2-bromo-2-chloro-1,1,1-trifluoroethane. Mixtures of halothane with air or oxygen are not flammable or explosive. With the exception of chromium, nickel, and titanium, most metals are corroded by halothane. The compound interacts with rubber and some plastics but not with polyethylene. Enflurane is 2-chloro-1,1,2-trifluoroethyl difluoro-methyl ether. It is a clear, colorless, nonflammable liquid with a mild sweet odor. It is extremely stable chemically. It does not attack aluminum, tin, brass, iron, or copper. Enflurane is soluble in rubber and this property may prolong induction and recovery. Isoflurane is 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether. The chemical and physical properties of isoflurane are similar to those of its isomer enflurane. It is not flammable in air or oxygen. Its vapor pressure is high, and delivery of safe concentrations necessitates the use of a precise vaporizer. Methoxyflurane is 2,2-dichloro-1,1-difluoroethyl methyl ether. It is a clear, colorless liquid with a sweet fruity odor. It is stable in the presence of soda lime and is nonflammable and nonexplosive in air or oxygen in anesthetic concentrations. It is very soluble in rubber. Sevofluorane, fluoromethyl-1,1,1,3,3,3-hexafluoroisopropyl ether, is a relatively new, nonflammable inhalational anesthetic agent. Nitrous oxide, N_2O, is a colorless gas without appreciable odor or taste. It is marketed in steel cylinders as a colorless liquid under pressure in equilibrium with its gas phase. Although nitrous oxide is not flammable, it supports combustion as actively as does oxygen when it is present in adequate concentrations with a flammable anesthetic. Nitrous oxide has relatively low solubility in blood the blood–gas partition coefficient λ; at 37°C it is 0.47 (Table 14.3).

14.3.1.2 Pharmacological Effects

Inhalational anesthetics currently used for general anesthesia include halothane, enflurane, isoflurane, methoxyflurane, and nitrous oxide. Nitrous oxide is a gas

TABLE 14.3 Partition Coefficients of Inhalational Anesthetic Agents

Anesthetic	Oil/Gas, λ	Blood/Gas, λ
Methoxyfluorane	970	12.0
Halothane	224	2.3
Enflurane	98	1.9
Isoflurane	99	1.4
Nitrous oxide	1.4	0.47

at normal ambient temperature and pressure, whereas the other four agents are volatile organic liquids. These agents share the common denominator of inducing rapid loss of consciousness that progresses to the absence of perception of all sensations or anesthesia. During general anesthesia produced with an inhalational agent, the depth of anesthesia varies directly with the partial pressure of anesthetic agent in the brain, and the rates of induction and recovery depend upon the rate of change of partial pressure in this tissue. The partial pressure of the anesthetic agent in the brain is always approaching that in arterial blood. The factors that determined the partial pressure of the anesthetic agent in the arterial blood and brain are (1) the concentration of the anesthetic agent in the inspired gas, (2) pulmonary ventilation delivering the anesthetic to the lungs, (3) transfer of the gas from the alveoli to the blood flowing through the lungs, and (4) loss of the agent from the arterial blood to all the tissues of the body.

14.3.1.3 Mechanism of Action

The molecular mechanism responsible for the anesthetic effect on inhalational anesthetics is related to their lipid solubility or hydrophobicity. Nuclear magnetic and electron paramagnetic resonance (NMR and EPR) studies indicate that inhalational anesthetic agents act by causing a local disordering of the lipid matrix (4–6). It is hypothesized that fluctuations of volume in biological membranes are sufficiently large to be important in the regulation of the structural state of membrane-bound proteins, namely, their state of aggregation; and, therefore, of their functional properties (7,8). As inhibitors of such fluctuations, anesthetics could readily influence the fluxes of ions, which are crucial determinants of neuronal excitability, or other functions of membranes that are determined by the proteins that function in the milieu of a dynamic lipid matrix (9).

14.3.1.4 Absorption and Elimination

When a constant partial pressure of anesthetic gas is inhaled, the corresponding partial pressure in arterial blood approaches that of the agent in the inspired mixture. for nitrous oxide the arterial partial pressure reaches 90% of the inspired pressure in about 20 min. With diethyl ether the same level is only attained after several hours. Although pulmonary ventilation influences the speed of induction

of anesthesia, it does not alter the ultimate depth of anesthesia; this depends on the final partial pressure of the anesthetic in brain, not the rate of change of that pressure. The solubility of the agent in blood is expressed as the blood/gas partition coefficient λ, which represents the ratio of anesthetic concentration in blood to anesthetic concentration in a gas phase when the two are in equilibrium. The λ value is as high as 12 for very soluble agents such as methoxyfluorane or diethyl ether, and as low as 0.47 for relatively insoluble agents such as nitrous oxide. The blood–gas partition coefficients for the commonly used inhalational anesthetics are given in Table 14.3.

When the inhalational agents are delivered by arterial blood to the tissues, the partial pressure rises in tissues to approach that in arterial blood. The rate at which a gas diffuses into tissues depends on (1) the solubility of the gas in the tissues, (2) the rate at which the gas is delivered to the tissues, and (3) the partial pressures of the gas in arterial blood and tissues. The solubility of the gas in the tissues is expressed as a tissue–blood partition coefficient, a concept analogous to the blood–gas partition coefficient discussed previously.

The major factors that affect the rate of elimination of the anesthetics are the same as those that are important in the uptake phase: pulmonary ventilation, bloodflow, and solubility in blood and in tissue. As ventilation with anesthetic-free gas washes out the lungs, the arterial blood pressure declines first, followed by that in tissues. Soon after elimination begins, the partial pressure in lung and blood falls to very low levels. Because of the very high flow to the brain, the partial pressure of the anesthetic decreases rapidly, accounting for the rapid awakening from anesthesia noted with relatively insoluble agents such as nitrous oxide. The agent persists for a longer time in tissues with lower bloodflow such as muscle, and for even longer times in fat tissue, where bloodflow is very low, and from which the agent is therefore slowly removed. The inhalational anesthetic agents are metabolized in the body to a variable extent. With most agents the amount metabolized is small. However, up to 15% of halothane, and 70% of methylflurane are metabolized to various intermediate compounds, and in some cases to ionized halogens (10). The importance of the metabolism of anesthetic agents is not in the termination of their action but in that the metabolites produced may be responsible for their toxic after effects. Additional small losses of anesthetic gases from the body occur by diffusion across skin and mucous membranes, and by means of urinary excretion of the agent or its breakdown products (10).

14.3.2 Gas Chromatographic Analysis

14.3.2.1 Sample Preparation

The method herein described is a modification of that reported by Yang et al. (11) Aliquots of 1.0 mL of 2.6 mg/mL 1,4-dioxane are transferred to a 1-mL headspace vial as an internal standard solution. The vial is sealed immediately with a rubber cap and an aluminum crimp seal. Aliquots of 100 μL of the plasma or urine sample are injected through the septum into a headspace vial using a

250-μL gastight syringe. The sample is then analyzed using the modified HS-GC-MS method described by Yang et al. (11).

14.3.2.2 Analytical Procedure

The GCMS system consists of a gas chromatograph fitted with a DB-5 capillary column (60 m × 0.25 mm i.d., 0.25 μm thickness), a mass detector, a headspace autosampler (HS850 from CE Instruments, Italy), and a computer using the Xcalibur program version 1.0. Helium is used as the carrier gas at a velocity of 40 cm/s. The split mode is used at a split rate of 1–30. Temperatures of the injection port and mass detector interface are set at 100 and 275°C, respectively. The temperature gradient of the gas chromatographic oven is programmed to initiate at 35°C for 3.5 min, then increased to 120°C at a rate of 40°C/min and hold at 120°C for 0.68 min. The completion of a temperature cycle takes 6.50 min. The data were acquired after a 2-min delay in both full scan (40–250 u) and selective-ion monitoring (SIM) modes. In the SIM mode, the following ions were selected: m/z 51, 69, and 149 for desfluorane; m/z 51, 69 and 181 for sevofluorane; m/z 51, 67, 117, and 149 for isofluorane; m/z 51, 67, and 117 for enflurane; m/z 67, 98, 117, and 178 for halothane; and m/z 55 and 88 for 1,4-dioxane. For quantification, the following ions were used: m/z 51 for desflurane; m/z 51, 69, and 181 for sevoflurane; m/z 51, 67, 117 for isoflurane and enflurane; m/z 67, 98, 117, and 178 for halothane; and m/z 55 and 88 for 1,4-dioxane.

14.3.2.3 Quantification of Inhalational Anesthetics

The retention times of five different inhalational anesthetics are shown in Table 14.4. Desfluorane is the first one to elute followed by sevoflurane, isoflurane, enflurane and halothane. The major ions, linearity range, and correlation coefficients are shown in Table 14.5.

The analysis results for all five anesthetics show excellent linear relationships ($r > 0.999$) within certain concentration ranges of the calibration standard. Both precision and accuracy results. The pharmacological concentrations of inhalational anesthetics are about 100 μg/mL. The lowest limit of detection range from 0.6 μg/mL for enflurane to 2.3 μg/mL for desflurane.

TABLE 14.4 Retention Times of Inhalational Anesthetics

Anesthetic	Retention Time (min)
Desflurane	2.85
Sevoflurane	3.10
Isoflurane	3.24
Enflurane	3.37
Halothane	3.88
1,4-Dioxane	5.65

TABLE 14.5 Quantification Limit and Linearity for the Analysis of Inhalational Anesthetics

Anesthetic	Major Ions (m/z)	Linear Range (μg/mL)	r Value
Desflurane	51,60,149	18.3–293	0.9991
Sevoflurane	51,69,131,181	19.0–304	0.9991
Isoflurane	51, 57, 117, 149	18.7–299	0.9994
Enflurane	51, 67, 117	19.0–303	0.9992
Halothane	67,98,117,128,178	23.3–373	0.9990

14.4 TRICYCLIC ANTIDEPRESSANTS

Imipramine, amitriptyline, their *N*-demethylatedmethyl derivatives, and other closely related compounds are the drugs currently most widely used for the treatment of major depression. Because of their structure, they are often referred to as "tricyclic" antidepressants. Their efficacy in alleviating major depression is well established, and support for their use in other psychiatric disorders is growing.

14.4.1 Pharmacological Considerations

14.4.1.1 Chemistry and Structure

In addition to the diabenzazepines, imipramine and desipramine, there are amitriptyline and its *N*-demethylated metabolite nortriptyline (dibenzocycloheptadienes) as well as doxepin, a dibenzoxepine, and potrityline. Additional structurally related agents approved for general use in the United States are trimipramine, a benzodiazepine; maptroline, containing an additional methylene bridge across the central six-carbon ring; and amoxapine, a dibenzoxazepine mix mixed neuroleptic and antidepressant properties. Since these agents all have a three-ring molecular core and produce therapeutic responses in most patients with major depression, the trivial name tricyclic antidepressants is used for this group.

14.4.1.2 Pharmacological Effects

Administration of therapeutic doses of a tricyclic antidepressant to depressed patients results in an elevation of the mood. About 2–3 weeks should be allowed for the antidepressant to exert its effect. For this reason, the tricyclic antidepressants cannot be prescribed on an as-needed basis. With some antidepressants, sedative or antianxiety effects may appear within a few days of treatment. The manner in which these agents relieve the signs of depression is not clear. However, manic excitement as well as euphoria and insomnia can be induced in some patients, contributing to the conclusion that antidepressant agents have clinically important mood-elevating actions.

14.4.1.3 Mechanism of Action

The administration of a tricyclic antidepressant produces an immediate reduction in the firing rate of neurons containing norepinephrine, and a decrease in their turnover rate. These changes are thought to be a consequence of blockade of the uptake of monoamines by neurons with a resultant increase in their action on presynaptic α_2-adrenergic receptors that serve to regulate the excitability of and transmitter release from monoaminergic neurons. Tricyclic antidepressants also act as antagonists at receptors for various neurohormones; these include moderate to high affinity at muscarinic cholinergic (12), α_1-adrenergic (13), and both H_1- and H_2-histaminergic receptors (14).

14.4.1.4 Absorption and Elimination

Imipramine and other tricyclic antidepressants are well absorbed after oral administration. Once absorbed, these lipophilic drugs are widely distributed. Their pharmacokinetics are similar to those of the phenothiazines. They are strongly bound to plasma protein and to constituents of tissues. The latter fact accounts for their large volumes of apparent distribution, which are typically 10–50 L/kg. The levels of these drugs in plasma correlate with satisfactory antidepressant responses at concentrations that range between 50 and 300 ng/mL. Toxic effects can be expected when their concentrations in plasma rise to 1 μg/mL or even less (15). The tricyclic antidepressants are oxidized by hepatic microsomal enzymes followed by conjugation with glucoronic acid. The major route of metabolism of imipramine is to the active product desipramine. Biotransformation of either compound occurs largely by oxidation to 2-hydroxy metabolites, which retain some ability to block the uptake of amines and may have particularly prominent cardiac depressant action (16). In contrast, amitriptyline and nortriptyline undergo preferred oxidation at the 10 position. The conjugation of hydroxylated metabolites with glucoronic acid extinguishes any remaining biological activity.

14.4.2 Gas Chromatographic Analysis

14.4.2.1 Sample Preparation

Aliquots of 1 mL of plasma or urine samples are mixed with 1 mL of 1 M NaHCO3 and 2 mL of distilled water and loaded onto a Sep-Pak cartridge. When blood is used, the mixture is centrifuged at 600 g for 8 min and the supernatant used for the next step. The sample solution is poured into the preconditioned cartridge at a flowrate of 5 mL/min. Sep-Pak cartridges are preconditioned with 10 mL of chloroform-2-propanol (9–1, v/v), 10 mL of acetonitrile, and 10 mL of distilled water. Then 10 mL of distilled water is added followed by 3 mL of chloroform-2-propanol (9–1, v/v) to elute the antidepressants. The eluate consists of an organic lower phase and an aqueous upper phase. The latter is discarded and the organic phase is evaporated to dryness. The residue is dissolved in 100 μL of methanol.

TABLE 14.6 Retention Times of Tricyclic Antidepressants

Antidepressant	Retention Time (min)
Amitrptyiline	8.90
Imipramine	25.3
Trimipramine	26.1
Chlorimipramine	32.3

14.4.2.2 Analytical Procedure

Aliquots of 1 μL of the sample extract dissolved in methanol are injected into a Shimadzu GC-15A instrument equipped with SID system with a fused-silica SPB-1 capillary column (30 m × 0.32 mm, i.d., and a film thickness of 0.25 μm, Supelco, Bellefonte, PA) in the splitless mode. Column temperature is 100−280°C at a rate of 6°C/min with an injection temperature of 200°C. SID conditions included a platinum emitter current of 2.2 A, an emitter temperature of 600°C, and a ring electrode bias voltage of +200 V with respect to the collector electrode.

14.4.2.3 Quantification of Tricyclic Antidepressants

The retention times corresponding to the antidepressants imipramine, amitriptyline, trimipramine, and chlorimipramine are shown in Table 14.6. Five nanograms of each were added to 1 mL of either urine, plasma, or blood and extracted with Sep-Pak cartridge, as indicated above. Recoveries are above 60%. Linear detector response is obtained between 10 and 80 pg in the injected volume (10−80 ng/mL). The lower limit of detection provides an extremely sensitive alternative to the standard thermoionic ionization detector (17).

14.5 ANTIEPILEPTIC DRUGS

14.5.1 Pharmacological Considerations

14.5.1.1 Chemistry and Structure

The useful antiepileptic agents belong to several chemical classes. Most of the drugs introduced before 1965 are closely related in structure to phenobarbital, the oldest member of this therapeutic class. These include the hydantoins, phenytoin, mephenytoin, and ethotoin; the deoxybarbiturate, primidone; the oxazolidinediones, trimethadione, and paramethadione; and the succinimides, ethosuximide, methsuximide, and phensuximide. The agents introduced after 1965 include the benzodiazepines clonazepan and clorazepate, an iminostilbene, carbamazepine, and a branched-chain carboxylic acid, valproic acid.

14.5.1.2 Pharmacological Effects

There are two general ways in which drugs might abolish or attenuate seizures: through effects on pathologically altered neurons of seizure foci to prevent or

reduce their excessive discharge, and through effects that would reduce the spread of excitation from seizure foci and prevent detonation and disruption of function of normal aggregates of neurons. Most of the antiepileptic drugs available exert their effect through the second mechanism, since all modify the ability of the brain to respond to various seizure-evoking stimuli.

14.5.1.3 Mechanism of Action

Antiepileptic drugs exert an stabilizing effect on excitable membranes of a variety of cells, including neurons and cardiac myocytes. They can decrease resting fluxes of Na^+ as well as Na^+ currents that flow during action potentials or chemically induced depolarization (18). The latter effects probably result from inhibition of voltage-sensitive Na^+ channels. Phenytoin-induced inhibition of Na^+ currents is dependent on both voltage and frequency and thus resembles the action of local anesthetics. As a result, antiepileptic drugs suppress episodes of repetitive neuronal firing that are induced by passage of intracellular current. Such effects can be achieved at concentrations of the drug below 10 μM. At concentrations in excess of 10 μM, phenytoin delays the activation of outward K^+ currents during action potentials in nerves, leading to an increased refractory period (19). Phenytoin can also reduce the size and duration of Ca^{2+}-dependent action potentials in cultured neurons at about 20 μM (20). On the other hand, the ability of the barbiturate phenobarbital to exert its anticonvulsant is thought to be mediated by producing a reduction in gamma-aminobutyric acid (GABA) and Ca^{2+}-dependent release of neurotransmitters.

14.5.1.4 Absorption and Elimination

The pharmacokinetics of hydantoins are markedly influenced by its limited aqueous solubility and by dose-dependent elimination. Phenytoin is a weak acid with a pK_a of $\sim$ 8.3; its aqueous solubility is limited, even in the intestine. On intramuscular injection, the drug precipitates at the injection site and is absorbed slowly and unpredictably. Absorption of phenytoin after oral ingestion is slow, and significant differences in bioavailability of oral pharmaceutical preparations have been detected. Peak plasma concentrations occur as early as 3 h after a single dose and last for about 12 h. Phenytoin is extensively bound to plasma proteins, mainly albumin. Fractional binding in tissues, including brain, is about the same as in plasma. Therefore, the apparent volume of distribution of phenytoin is about 0.6 L/kg. Less than 5% is excreted unchanged in urine. The remainder is metabolized primarily in the hepatic endoplasmic reticulum. The major metabolite, the parahydroxyphenyl derivative, is inactive. Oral absorption of phenobarbital is complete but somewhat slow. Peak concentrations in plasma occur several hours after a single dose. It is 40–60% bound to plasma proteins and bound to a similar extent in tissues including brain. The volume of distribution is about 0.5 L/kg. The pK_a of phenobarbital is 7.3, and up to 25% of a dose is eliminated by pH-dependent renal excretion of the unchanged drug. The remainder is inactivated by hepatic microsomal enzymes.

The deoxybarbiturate, primidone, is rapidly and almost completely absorbed after oral administration. Peak plasma concentrations are reached within 3 hours

after ingestion. Primidone is converted to two active metabolites, phenobarbital and phenylethylmalonamide (PEMA). Primidone and PEMA are bound to plasma proteins to a much lesser extent than phenobarbital. Approximately 40% of primidone is excreted unchanged in the urine, unconjugated PEMA, and phenobarbital. The succinimide, ethosuximide, is also rapidly and completely absorbed after oral administration, reaching plasma peak concentrations after 3 h of administration. It is not significantly bound to plasma proteins, and the apparent volume of distribution averages 0.7 L/kg. Valproic acid is rapidly and completely absorbed after oral administration. Peak concentration in plasma is observed in 1–4 h. The apparent volume of distribution of valproate is 0.2 L/kg. Its extent of binding to plasma proteins is about 90%. Although concentrations of valproate in the central nervous system suggest equilibration with free drug in the blood, there is evidence for carrier-mediated transport of valproate both into and out of the central nervous system. The majority of the drug is converted to the conjugate ester of glucoronic acid while β-oxidation in the mitochondria accounts for the remainder. Some of these metabolites, notably 2-propyl-2-pentenoic acid and 2-propyl-4-pentenoic acid, are nearly as potent anticonvulsant agents as the parent compound. However, only 2-en-valproic acid accumulates in plasma and brain to a significant extent.

14.5.2 Gas Chromatographic Analysis

14.5.2.1 Sample Preparation

The methodology herein described is a modification of that reported by Volmut et al. (21). A 500-μL aliquot of plasma or urine is mixed with 100 μL of 0.5 M HCl in a 1.5-mL polypropylene microvial and a 10-μL aliquot of the internal standard solution mixture in methanol containing valproic acid, ethosuximide, phenobarbital, primidone, carbamazepina, and phenytoin added. A 2-mL disposable polyethylene syringe (9 mm i.d.) is packed with 200 mg of Silipor C18 and the column preconditioned with 1 mL of methanol followed by 1 mL of distilled water or 0.5 M HCl. The pretreated sample is poured onto the column and allowed to flow through. The column is then rinsed with two 1-mL portions of water and dried under vacuum. The drugs are eluted with 1 mL of methanol. A 50-μL volume of a 0.05 M KOH solution in methanol is added to 1 mL of eluate and the solvent is evaporated to dryness. Addition of KOH to the evaporation step prevents loss of volatile antiepileptic drugs from the sample. The residue is dissolved in 50 μL of a 0.05 M solution of HCl in methanol. The internal standard, caprilic acid or 5-phenylhidantoin, is added to the samples at a concentration of 20 μg/ mL

14.5.2.2 Analytical Procedure

A HP-5790A gas chromatograph equipped with a split/splitless capillary inlet system and a flame ionization detector are used. A fused-silica capillary column with crosslinked 5% phenylmethylsilicone gum phase HP-5, 25 m × 0.20 mm i.d., 0.33 μm film thickness, is used. Nitrogen is used as a carrier gas at an inlet pressure of 100 kPa. The oven is operated isothermally at 60°C for 0.5 min after injection, heated at 30°C/min to 200°C, then at 60°C/min to 250°C, and then

TABLE 14.7 Recoveries for Antiepileptic Drugs

Substance	Recovery (%)
Valproic acid	85.1
Caprilic acid	78.0
Ethosuximide	31.5
Phenobarbital	96.0
Primidone	75.0
Carbamazepina	88.0
Phenytoin	100.0

TABLE 14.8 Retention Times for Antiepileptic Drugs

Substance	Retention Time (min)
Valproic acid	6.1
Caprilic acid	6.4
Ethosuximide	7.1
Hexobarbital	13.1
Phenobarbital	14.2
Primidone	20.2
Carbamazepina	23.1
Phenytoin	23.5
MMPH	27.6

held at 250°C for 10 min. Aliquots of 2 μL are injected in the split mode at a split ratio of 1−20 and a septum purge rate of 1 mL/min. The temperatures of injector and detector are 240 and 300°C, respectively. Hexobarbital is used as an internal standard during the chromatographic run, but it is not added to serum samples because of the presence of a peak in the biological matrix with a very close retention time (Table14.7).

14.5.2.3 Quantification of Antiepileptic Drugs

The percent recoveries relative to 5-(4-methylphenyl)-5-phenylhydantoin for the various antiepileptic drugs using this methodology are shown in Table 14.7. The retention times for the various antiepileptic drugs are shown in Table 14.8. The chromatographic run-to-run reproducibility for different concentrations of antiepileptic drugs is calculated by the relative standard method using MPPH as the internal standard and ranges from 7 to 9.9%. Analysis of these antiepileptic drugs can be accomplished in less than 30 min.

14.6 BLOOD ALCOHOL

Alcoholic beverages have been used since the dawn of history, beginning with fermented beverages of relatively low alcohol content. When the Arabs introduced the alambique in Europe in the Middle Ages as a means of distilling

alcohol, the alchemists believed that alcohol was the long-sought elixir of life. Alcohol was therefore held to be a remedy for practically all diseases, as indicated by the term "whisky" (Gaelic: *usquebaugh*, meaning "water of life"). It is now recognized that the therapeutic value of ethanol is extremely limited and that chronic ingestion of excessive amounts is a major social and medical problem. Methanol (methyl alcohol or wood alcohol) is a common industrial solvent. It is also used as an antifreeze fluid, a solvent for shellac and some paints and varnishes, and a component of paint removers. As an adulterant, it renders unpotable and tax-free the ethanol that is used for cleaning, paint removal and other purposes. Isopropanol, used for rubbing alcohol, in hand lotions, and in deicing and antifreeze preparations, is occasionally the cause of accidental poisoning.

14.6.1 Pharmacological Considerations

14.6.1.1 Pharmacological Effects

Alcohol is primarily a continuous depressant of the central nervous system. As with other depressants, the apparent stimulation results from depression of inhibitory control mechanism in the brain. The first mental processes to be affected are those that depend on training and previous experience. Memory, concentration, and insight are dulled and then lost. Confidence abounds, the personality becomes expansive and vivacious, and uncontrolled mood swings and emotional outbursts may be evident. The psychic changes are accompanied by sensory and motor disturbances. As intoxication becomes more advanced, a general impairment of nervous function occurs and a condition of general anesthesia ultimately prevails. Methanol cause less inebriation than ethanol. Symptoms of methanol poisoning include headache, vertigo, vomiting, severe upper abdominal pain, blurring of vision, and hyperemia of the optic disk. The most pronounced laboratory finding is severe metabolic acidosis as a result of the oxidation of methanol to formic acid (22,23). Visual disturbances, the most distinctive aspect of methanol poisoning in humans, become evident soon after the onset of acidosis. The final result is bilateral blindness, which is usually permanent. Like ethanol and methanol, isopropanol is a CNS depressant, but it does not produce retinal damage or acidosis as does methanol. Isopropanol produces a more prominent gastritis, with pain, vomiting, and hemorrhage. As with the other alcohols, hemodialysis is useful for removing isopropanol from the body (24).

14.6.1.2 Mechanism of Action

Since Chin and Goldstein in 1981 (25) reported the membrane-fluidizing effects of ethanol, a number of investigators have shown a correlation between the degree of intoxication and the extent of ethanol-induced disordering of membranes (26). These disordering effects, however, occur in regions or domains of biological membranes reflecting the nonuniform distribution of various phospholipids and cholesterol within the lipid bilayer. Moreover, the hydrophobic domains of membrane-bound proteins represent additional targets for ethanol and other aliphatic agents. Attention has also focused on the capacity of ethanol

to augment GABA-mediated synaptic inhibition as well as fluxes of chloride. Such effects, as well as the sedative-ataxic actions of ethanol are inhibited by bicuculline, a specific antagonist of GABA-ergic receptors. All of these properties closely resemble those of the anesthetic barbiturates, and they are shared by other aliphatic alcohols and a variety of anesthetic agents.

14.6.1.3 Absorption and Elimination

Ethanol is rapidly absorbed from the stomach, small intestine, and colon. The time from the last drink to maximal concentration in plasma usually ranges within 30–90 min. Vaporized ethanol can be absorbed through the lungs, and fatal intoxication has occurred as a result of its inhalation. Between 90 and 98% of the ethanol that enters the body is completely oxidized. The metabolism of ethanol follows zero-order kinetics. That is, it proceeds linearly with time and the rate of oxidation is independent of its concentration. The amount of ethanol metabolized per unit of time is roughly proportional to body weight. In the adult, the average rate at which ethanol can be metabolized is about 30 mL in 3 h. the oxidation of ethanol occurs chiefly in the liver initiated by alcohol dehydrogenase, which is a zinc-containing enzyme that utilizes NAD as the hydrogen acceptor. The product, acetaldehyde, is converted to acetyl-CoA, which is then oxidized through the Krebs cycle or utilized in the synthesis of cholesterol, fatty acids, or other tissue constituents. It is generally agreed that threshold effects of intoxication appear when the concentration in plasma is 20–30 mg/100 mL (0.02–0.03%). More than 50% of persons are grossly intoxicated when the concentration is 150 mg/100 mL. The average concentration in fatal cases is about 400 mg/100 mL. The absorption of methanol and ethanol are similar. In addition, methanol is metabolized in humans by the same enzymes that metabolize ethanol: alcohol dehydrogenase and aldehyde dehydrogenase, to form formaldehyde and formic acid. (27). Oxidation of methanol, like that of ethanol, proceeds at a rate that is independent of its concentration in plasma. However, this rate is only one-seventh that of ethanol, and complete oxidation and excretion usually require several days.

14.6.2 Gas Chromatographic Analysis

14.6.2.1 Sample Preparation

The method described herein for the analysis of volatile organics is a modified procedure of that described by Schuberth (28). Aliquots of 10–20 mL of blood are obtained from the test subjects with the use of a Vacutainer and collected into 10-mL tubes containing 15 mg of ethylenediaminotetraacetic acid (EDTA) and 100 mg of NaF as anticoagulant and preservative, respectively. The samples are then stored at 4°C. Aliquots of 1.5 mL of blood are then added to a headspace vial containing 1.8 g of NaCl. Headspace extraction is done at a bath temperature of 50°C and an equilibrium time of 30 min.

14.6.2.2 Analytical Procedure

A 30-m × 0.25-mm DB-WAX capillary column, coated with 0.25 μm of polyethylene glycol, is used. The valve/loop temperature is 54°C. Injection time

TABLE 14.9 Retention Times and Limit of Detection of Volatile Organics

Volatile	Retention Time (min)	Limit of Detection (nmol/L)
Acetaldehyde	1.37	0.15
2-Propanone	2.04	0.015
Ethyl acetate	2.39	0.0005
2-Butanone	2.48	0.006
Methanol	2.49	1.50
2-Propanol	3.16	0.06
Ethanol	3.21	0.70
2-Butanol	5.13	0.03
1-Propanol	5.31	0.03

is 1 s, and injection volume is 1 mL. The temperature program is of an initial temperature of 40°C held for 4 min and then increased at a rate of 10°C/min to a final temperature of 150°C. An ion trap detection system is utilized, with electron impact as the ionization mode (50–80 eV) (see also Chapter 16).

14.6.2.3 Quantification of Volatile Organics

The retention times and limits of detection of the various volatile organics in plasma are shown in Table 14.9. No volatile organics, with the exception of acetone, are normally found in blood. Ingestion of alcoholic beverages with more than 10 pmol of ethanol per liter results in the detection of ethanol, acetaldehyde, 2-propanone, ketones, and esters.

14.7 DRUGS OF ABUSE

Every society in recorded history has used drugs that produce effects on mood, thought, and feeling. Moreover, there were always a few individuals who digressed from custom with respect to the time the amount, and the situation in which these drugs were to be used. Thus, both the nonmedical use of drugs and the problem of drug abuse are as old as civilization itself. Drugs of abuse include, alcohol, cocaine, amphetamines, nicotine, tobacco, cannabinoids (marihuana), lysergic acid diethylamide (LSD), arylcyclohexylamines, and barbiturates. In the United States two thirds of all adults use alcohol occasionally, and at least 12% of the users can be considered “heavy drinkers.” The lifetime dependence or abuse is estimated at about 13%, with the risk for men far higher than women. It has been estimated that 20 million people in the United States have used cocaine. In 1988, 5% of young adults reported using cocaine and 2% reported using a stimulant other than cocaine during the 30 days prior to the survey. The increased use of cocaine by injection of its salts and by inhalation of the free alkaloid (“crack”) has been responsible for many serious toxic reactions and escalating crime rates.

Per capita consumption of cigarettes in the United States has been declining since 1973. In 1988, 27% of adults were still smokers, but only 19% of high school seniors were regular smokers. Smokeless tobacco (snuff and chewing tobacco) is now used by 8% of young men. The rationale for considering use of tobacco as a form of drug dependence is presented by Jaffe in 1990 to the Surgeon General. Marijuana, also known as "grass," "weed," "pot," "Mary Jane," and "reefer," is still by far the most commonly used illicit drug in the United States. About 55% of young adults report some lifetime experience with the drug. There is, however, a downward trend. Among high school seniors, the use of marihuana in the month before survey has declined steadily from 37% in 1978 to 18% in 1988. The incidence of daily use among high school seniors is currently reported to be 2.7%.

14.7.1 Pharmacological Considerations

14.7.1.1 Pharmacological Effects

The most relevant pharmacological effect(s) induced by drugs of abuse, in addition to their particular effects on the target tissue, is the physical dependence. Physical dependence has been studied after long-term administration of opioids, ethanol, barbiturates, related hypnotics, benzodiazepines, amphetamines, cocaine, cannabinoids, phencyclidine, and nicotine. The withdrawal symptoms associated with many of these agents are generally characterized by rebound effects in those physiological systems that were initially modified by the drug. For example, amphetamines and cocaine alleviate fatigue, suppress appetite, and elevate mood; withdrawal from these drugs is characterized by lack of energy, increase appetite, and depression.

14.7.1.2 Mechanism of Action

A number of mechanisms have been proposed to explain the changes induced by drugs of abuse, some of which help to account for the observation that physical dependence is generally accompanied by tolerance and that the two phenomena develop and decay at about the same rate. However, there is growing evidence that for some drugs, notably ethanol, it is possible to distinguish the mechanism responsible for tolerance from those responsible for physical dependence. The mechanisms responsible for opioid-induced physical dependence are among the most thoroughly studied. Although an increase in the number of opioid receptors follows a long-term administration of opioid antagonists, a continuous administration of opioid agonists does not change the number of affinity of such receptors in the CNS. However, adaptive changes in the second messenger systems that are altered by stimulation of opioid receptors can be detected. For example, in some areas of the brain the effects of opioids include inhibition of adenyl cyclase, an action mediated by the inhibitory guanine nucleotide-binding regulatory protein Gi; this effect is shared by α_2-adrenergic agonists. The long-term administration of morphine causes a compensatory increase in adenylate cyclase activity that may be partially responsible for the rebound excitability of neurons in those areas of the brain that typically occurs during opioid withdrawal. The common intracellular mechanism helps to explain the utility of clonidine and other α_2-adrenergic agonists in suppressing some elements of the opioid withdrawal syndrome.

14.7.2 Gas Chromatographic Analysis

14.7.2.1 Sample Preparation

The method described here corresponds to a modification of that reported by Williams et al. (29). A 10-ng/mL Toxiclean drug mixture (Alltech, Chicago, IL) containing amphetamine, methamphetamine, butabarbital, amobarbital, meperidine, pentobarbital, secobarbital, glutethimide, phencyclidine, methaqualone, methadone, cocaine, amitrptyline, imipramine, doxepin, desipramine, pentazocine, codeine, and heroin is employed.

14.7.2.2 Analytical Procedure

Plasma or urine samples were analyzed on a Falsh GC equipped with a flame ionization detector. Separations are performed using 6-m $\times$ 0.32-mm RTX-1 (Restek, Bellefonte, PA) fused-silica capillary column with a 0.1 μm film thickness. Approximately 1 μL of sample is injected into the injection port. Injection is performed in the split mode with a split vent flow of 70 mL/min. The injector temperature is set at 250°C, the main oven heater at 300°C, and the detector at 325°C. Helium is used as the carrier gas at a flowrate of 4.47 mL/ min.

14.7.2.3 Quantification of Drugs of Abuse

The first eluting peak was amphetamine with a retention time of 19 s and the last oxycodone with a retention time of 90 s (Table 14.10). The average percent relative standard deviation (%RSD) for the retention times over all the analytes was 0.580%. Repeatability for 10 runs over a 2-day period was 0.629%. This method

TABLE 14.10 Retention Times of Drugs of Abuse

Drug	Retention Time (s)
Amphetamine	19
Methamphetamine	21
Butabarbital	41
Amobarbital	43
Meperidine	45
Pentobarbital	46
Secobarbital	47
Glutethimide	48
Phencyclidine	51
Phenobarbital	53
Methadone	65
Methaqualone	66
Amitrptyline	67
Cocaine	68
Imipramine	70
Desipramine	72
Pentazocine	73
Codeine	80
Heroin	90

gives excellent retention time reproducibility with little day-to-day variation in retention times (an essential requirement if retention times are to be sued by themselves for peak identification in routine screening by such fast GC methods like the one described herein).

14.8 PROSTAGLANDINS

Prostaglandins are membrane-derived lipids which are formed from certain polyunsaturated fatty acids in response to diverse stimuli. Since prostaglandins principally derive from arachidonic acid and therefore, have a twenty carbon backbone, they are also designated eicosanoids (from the greek, *eicosa*, which means twenty).

14.8.1 Pharmacological Considerations

14.8.1.1 Chemistry and Structure

Prostaglandins are derived from 20-carbon essential fatty acids that contain three, four, or five double bonds: 8,11,14-eicosatrienoic acid (dihomo-γ-linolenic acid); 5,8,11,14-eicosatetraenoic acid (arachidonic acid), and 5,8,11,14,17-eicosapentaenoic acid. Structurally, all prostaglandins have a "hairpin" configuration and are composed of a cyclopentanone nucleus with two sidechains. They are derived from the hypothetical structure, prostanoic acid. Primary prostaglandins contain a 15-hydroxy group with a double bond at carbon 13. Each group of prostaglandins is allocated a letter, A, B, C, D, E, F, G, H, or I, that denotes particular functional groups in the cyclopentane ring. The degree of unsaturation of the sidechains is indicated by the subscript numeral after the letter; thus PGE_1, PGE_2, and PGE_3 have one, two, and three double bonds, respectively. A description of the stereochemistry at position 9 in the cyclopentanone ring is denoted by the subscript α or β; thus the configuration of $PGF_{2\alpha}$ has the orientation of the 9-hydroxyl moiety oriented below the plane of the ring. $PGF_{2\beta}$ (the inactive isomer of $PGF_{2\alpha}$) has the 9-hydroxyl group oriented above the plane of the ring.

14.8.1.2 Pharmacological Effects

In most species, including humans, and in most vascular beds, the PGEs are potent vasodilators. The dilatation appears to involve arterioles, precapillaries, sphincters, and postcapillary venules. Similarly, PGD_2 causes also vasodilatation in most vascular beds. An exception is the pulmonary circulation in which PGD_2 causes only vasoconstriction. Responses to $PGF_{2\alpha}$ vary with species and vascular bed. It is a potent vasoconstrictor of both pulmonary arteries and veins in humans (30). Systemic blood pressure generally falls in response to PGEs, and bloodflow to most organs, including the heart, mesentery, and kidney, is increased. Prostaglandins contract or relax many smooth muscles besides those of the vasculature. In general, PGFs and PGD_2 contract and PGEs relax bronchial

and tracheal muscle. Asthmatic individuals are particularly sensitive to $PGF_{2\alpha}$ which causes intense bronchospasm. PGEs relax the uterine smooth muscle, while PGFs induce contraction. The contractile response is most prominent before menstruation, whereas relaxation is greatest at midcycle. Uterine strips from pregnant women are uniformly contracted by PGFs and by low concentrations of PGE_2. Prostaglandins are widely used to induce midtrimester abortion.

14.8.1.3 Mechanism of Action

The diversity of the effects of prostaglandins is explained by the existence of a number of distinct receptors that mediate their actions. The receptors have been named for the natural prostaglandin for which they have the greatest apparent affinity and have been divided in five main types, designated DP (PGD), FP (PGF), IP (PGI_2), and EP (PGE). The last one has been subdivided into EP_1 (smooth-muscle contraction) and EP_2 (smooth-muscle relaxation). As with many other receptors, the prostaglandin receptors are coupled to effector mechanisms through G proteins (31). The second messenger systems, adenyl cyclase and protein kinase C, have been implicated in the action of prostaglandins. PGE antagonizes the lipolytic actions of epinephrine and the effects of antidiuretic hormone at least in part by inhibition of adenyl cyclase.

14.8.1.4 Absorption and Elimination

About 95% of infused PGE_2 is inactivated during one passage through the pulmonary circulation. Because of the unique position of the lungs between the venous and the arterial circulation, the pulmonary vascular bed constitutes and important filter for prostaglandins that act locally prior to their release into the venous circulation from endogenous sources. Several enzymatic catabolic reactions are responsible for the metabolization of prostaglandins. These involve the oxidation of the prostaglandin molecule by prostaglandin 15-OH dehydrogenase, reduction of the 15-keto group to produce the 13,14-dihydro derivative by prostaglandin Δ^{13}-reductase, and β and ω oxidation of the side chains. The degradation of PGI_2 apparently begins with its spontaneous hydrolysis in blood to 6-keto-$PGF_{1\alpha}$.

14.8.2 Gas Chromatographic Analysis

14.8.2.1 Sample Preparation

The method hereind described corresponds to that reported by Wubert et al. (32). Plasma and urine samples were collected in vials containing 0.01% butylated hydroxytoluene to prevent oxidation, immediately frozen and stored at $-20^{\circ}C$ until analysis. Samples are spiked with 2.0 ng of $[^2H_4]$11-dehydrothromboxane B_2, 0.2 ng of $[^2H_3]$2,3-dinor-6-keto-prostaglandin F_{1a}, and 2.0 ng of $[^2H_4]$prostaglandins E_2, D_2, and $F_{2\alpha}$ as internal standards in ethanolic solution. To this was added 0.5 mL of 2-propanol. As tetradeuterated standards of isoprostanes are not commercially available at present, $[^2H_4]$prostaglandin $F_{2\alpha}$ serves as a substitute internal standard for its stereoisomeric isoprostanes. A

Chromabond C18ec cartridge is preconditioned with 12 mL of methanol, 6 mL of distilled water, and 6 mL of 0.05 N formic acid, loaded with the sample, and washed with 8 mL of 1 N formic acid/acetonitrile (3–1, v/v) and 4 mL of distilled water. The cartridge is dried by blowing nitrogen through it for 15 min and then eluted with 4 mL of methanol into a derivatization vial. The eluate is dried under nitrogen and the residue rinsed to the bottom of the tube with 500 μL of *tert*-butyl methyl ether and dried with nitrogen. The sample is redissolved in 100 μL of ethyl acetate/concentrated formic acid (9–1, v/v), activated at 45°C for 30 min, and dried under nitrogen. The samples were derivatized with 50 μL of 0.5 g of methoxyamine HCl in 9.5 mL of *N*,*N*-dimethylformamide at 45°C for 30 min and dried under nitrogen. The sample was redissolved in 50 μL of acetonitrile, 20 μL of *N*,*N*-diisopropylethylamine, and 20 μL of 1 g of pentafluorobenzylbromide in 3 mL of acetonitrile, allowed to react at 45°C for 25 min, and dried under nitrogen. The sample is taken up again in 50 μL of bis(trimethylsilyl)trifluorocetamide (BSTFA), incubated at 45°C for 2 h, and left at room temperature overnight.

14.8.2.2 Analytical Procedure

Aliquots of 5 μL of the sample is injected using a septumless Gerstel CIS3 cold injection system kept at 60°C for 6 s (solvent split) and then heated to 300°C at 10°C/s (splitless). The DB5MS capillary column is directly connected to the ion source. The initial column temperature of 100°C is maintained for 2 min. The column is than heated to 250°C in 7 min, then to 300°C at 2°C/min and kept there for 10 min. The transfer line and ion source are maintained at 300°C and 150°C, respectively. Helium is used as the carrier gas at a flowrate of 1 mL/min. The mass spectrometer is operated in the negative-ion, chemical ionization mode, utilizing methane as the reagent gas. The electron energy used is 70 eV and the filament current 0.2 mA. The masses used for quantification are the following: *m/z* 586 (589) for 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$ and its deuterated analog, respectively; *m/z* 569 (573) for prostaglandin $F_{2\alpha}$ its tetradeuterated analog, and its stereoisomeric isoprostanes 8-epi, 9-β-, and 11-β-$PGF_{2\alpha}$; *m/z* 524 (528) for prostaglandins D_2 and E_2; and *m/z* 511 (515) for 11-dehydrothromboxane B_2 (see Table 14.11).

TABLE 14.11 Ion Masses for Prostaglandins and Isoprostanes

Drug	Major Ion
2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$	586
11-dehydro-TXB_2	511
$PGF_{2\alpha}$	569
8-epi-$PGF_{2\alpha}$	569
9-β-$PGF_{2\alpha}$	569
11-β-$PGF_{2\alpha}$	569
PGD_2	524
PGE_2	524

14.8.2.3 Quantification of Prostaglandins

An acceptable signal-to-noise ratio is obtained down to the low picogram range of endogenous concentrations. The intrassay variation is below 7% for 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$, 11-dehydro-TXB_2, and $PGF_{2\alpha}$. For 8-epi-$PGF_{2\alpha}$, 11-β-$PGF_{2\alpha}$, and PGE_2 was below 15%. The interassay coefficient of variation was below 12% for 2,3-dinor-6-ketoprostaglandin $F_{1\alpha}$, 11-dehydro-TXB_2, and $PGF_{2\alpha}$, and below 18% for 8-epi-$PGF_{2\alpha}$, 11-β-$PGF_{2\alpha}$, PGD_2, and PGE_2.

14.9 STEROIDS

Steroids are a subclass of lipids that contain a basic skeletal structure of four fused rings referred to as *perhydrocyclopentanophenanthrene*. Steroids comprise a subcategory of chemical compounds that form part of a large family of substances that include rubber; guttapercha; the phytol sidechain of chlorophyll; numerous fragrant oils; turpentine hydrocarbons; carotenoids; vitamins A, E, and K; and cholesterol. The cholesterol, in turn, is converted into bile acids, steroid hormones, and vitamin D. What all these substances have in common is that they are formed by the polymerization of an activated five-carbon isoprene unit. The activated isoprene unit, isopentenyl pyrophosphate, is derived from acetyl-CoA and is the building block precursor of the various steroids. Cholesterol is formed by the polymerization of six activated isoprene units to form squalene, which contains 30 carbon atoms. The final stage in cholesterol biosynthesis involves cyclization, requires molecular oxygen, and results in the eventual removal of three methyl groups. Cholesterol has the distinction of being the first isopentenoid isolated in pure form, and from it the generic term “steroid” is derived.

14.9.1 Pharmacological Considerations

14.9.1.1 Chemistry and Structure

The most common natural occurring steroids are listed in Table 14.12.

14.9.1.2 Mechanism of Action

The classic steroid hormones are estrogens, progesterone, androgens, glucocorticoids, mineralocorticoids, and vitamin D. These are potent hormones that regulate the developmental and physiologic functions of female phenotype (estrogen), pregnancy (progesterone), male phenotype (androgens), metabolism and stress responses (glucocorticoids), salt and water balance (mineralocorticoid), and calcium metabolism (vitamin D). To accomplish this task, the steroid hormones must bind and activate a group of specific gene-regulatory molecules called *receptors*. These receptors are proteins that are present in cells in low amounts but bind steroid hormones specifically and very tightly. The hormones are secreted from their respective endocrine glands into the bloodstream, where they circulate, mostly bound (95%) to plasma transport proteins, which provide a reservoir for steroid supply to cells. Free steroid enters the cell and binds to inactive receptors

TABLE 14.12 Trivial Names of Steroids

Trivial Name	Systematic Name
Cholesterol	5-Cholesten-3β-ol
Androstenedione	4-Androstene-3, 17-dione
Testosterone	17β-Hydroxy-4-androsten-3-one
Androsterone	3α-Hydroxy-5α-androstan-17-one
Etiocholanolone	3α-Hydroxy-5β-androstan-17-one
Estrone	3-Hydroxy-1,3,5(10)-estratrien-17-one
Estradiol	1,3,5,(10)-Estratriene-3, 17β-diol
Estriol	1,3,5,(10)-Estratriene-3, 16α,17β,-triol
Pregnenolone	3β-Hydroxy-5-pregnen-20-one
Progesterone	4-Pregnene-3, 20-dione
Pregnanediol	5β-Pregnane-3α, 20α-diol
Cortisone	17α-21-Dihydroxy-4-pregnene-3,11,20-trione
Cortisol	11β-17α-21-Trihydroxy-4-pregnene-3,20-dione
Aldosterone	11β, 21-Dihydroxy-3,20-dioxo-4-pregnen-18-al

in either the cytoplasmic or nuclear compartments. On complexing with hormone, the receptor undergoes an allosteric conformational change into an active form capable of affecting nuclear gene transcription.

14.9.1.3 Absorption and Elimination

When an steroid enters the blood compartment and flows through each tissue in the body, a certain amount of the steroid will be removed or extracted. The *metabolic clearance rate* (MCR) has been defined as the volume of blood that has been completely cleared of a substance per unit time (L/day). The rate of clearance of a steroid from the blood of the whole body is the sum of the clearance rates for each tissue or organ, and it is this overall value that is termed MCR. The liver is the principal tissue for removing steroids from blood, and it is possible to directly determine the hepatic clearance rate. The MCR minus the hepatic clearance rate indicates how much of the total clearance has occurred in extrahepatic tissue. Since hepatic bloodflow is about 1500 L/day, a steroid with a MCR in excess of this value indicates that tissue other than the liver is extracting the steroid. The binding of steroids to specific plasma proteins such as testosterone-estradiol-binding globulin and cortisol-binding globulin or transcortin will suppress peripheral metabolism. This is reflected in relatively low MCRs for testosterone and cortisol; an increase in the level of a specific steroid-binding plasma protein can further decrease the MCR of the specifically bound steroid. Many unconjugated steroids bind to albumin, but unlike the specific binding, the albumin binding is of relatively low affinity whereas MCRs are relatively high, indicating that the binding of steroids to plasma albumin has little effect on metabolism. Steroids are inactivated primarily in the liver, and the inactive metabolites excreted in the urine are conjugated generally as sulfate esters or β-glucuronates.

14.9.2 Gas Chromatographic Analysis

14.9.2.1 Sample Preparation

The procedure herein described corresponds to that reported by Hamalainen et al. (33). The pH of the urine sample is adjusted to pH 3 with 1 M acetate buffer and the steroids extracted with Sep-Pak C18 cartridges. The steroids are eluted with 5 mL of methanol, distilled water added to the eluate to obtain a 70% water–methanol solution (v/v), and the sample added to a DEAE-Sephadex anion exchange column. Steroid conjugates are separated into free, monoglucoronide, monosulfate, and double-conjugate fractions on DEAE-Sephadex anion exchange columns (5 mm × 3 cm, packed in 70% methanol, v/v). The first 10 mL fraction of the eluate contains the free steroids. The weak organic acids and colored substances are eluted with 10 mL of 0.2 M acetic acid, monoglucoronides with 15 mL of 0.4 m formic acid, monosulfates with 15 mL of formic acid–potassium formate, and double conjugates with 15 mL of 0.3 M lithium chloride plus 0.1 M formic acid in 70% methanol. The glucoronide conjugates are hydrolyzed overnight at 39°C in 5 mL of 0.1 M acetate buffer (pH 6.8) containing 5 U of *Escherichia coli* β-glucoronidase, and the free steroids released extracted with Sep-Pak C18 cartridges, as described above. Steroid mono and disulfate conjugates are desalted with Sep-Pak C18 cartridges and the solvolysis of sulfate moieties allowed to proceed in a mixture of 300 μL of dimethylformamide and 5 μL of 6 N HCl in 3 mL dichloromethane overnight at 39°C. After solvolysis, the free steroids are purified on a DEAE-Sephadex anion exchange column in the acetate form, as described previously. After hydrolysis or solvolysis, the carbonyl groups of neutral steroids are derivatized with 4% methoxyamine HCl in pyridine for 2 h at 80°C. This protection reaction is necessary for quantitative recovery of the polar corticosteroid metabolites, especially for 3α-, 17α, 21-trihydroxy-5β-pregnan-11,20-dione. After methoximation, pyridine is evaporated to dryness, 0.5 mL of distilled water added, and the steroids extracted twice with 3 mL of ethylacetate. The combined organic phases are evaporated to dryness, dissolved in 0.5 mL of methanol, and applied to a 0.5 × 1.5-cm DEAE-Sephadex A-25 anion exchange column in the free base form. Neutral steroids are eluted with 3 mL of methanol and separated from estrogens and other phenolic steroids. One-tenth of the DEAE-OH column eluate is taken for recovery determination. Stigmasterol is added as an internal standard to each fraction and the steroids converted to their TMS and *O*-methoxime-TMS derivatives using overnight silylation with trimethylsilylimidazole at 80°C. The derivatives are purified by Lipidex 5000 microcolumns (5 mm × 2.5 cm) using hexane-pyridine-HMDS (98–1–1, v/v/v) as the eluent. The eluent is then evaporated to dryness, the residue redissolved in hexane, and subsequently analyzed by GCMS.

14.9.2.2 Analytical Procedure

A Perkin Elmer Sigma-1 gas-chromatographic system is used, equipped with a 25-m BP-1 bonded-phase column utilizing a flame ionization detector and hydrogen as the carrier gas at a flowrate of 2 mL/min. Analyses are carried out in the splitless mode using a two-stage program from 100 to 220°C (25°C/min) and 220

TABLE 14.13 Retention Times of Steroids

Steroid	Relative Retention Time
3α-Hydroxy-5α-androsten-17-one	0.39
3α-Hydroxy-5β-androsten-17-one	0.40
5-Androstene-3β-17α-diol	0.41
3β-Hydroxy-5α-androsten-17-one	0.44
3β-Hydroxy-5α-androstan-17-one	0.44
5-Androstene-3β-17β-diol	0.44
3α-Hydroxy-5α-androstane-11,17-dione	0.46
3α-Hydroxy-5β-androstane-11,17-dione	0.46
3β,7α-Dihydroxy-5-androstan-17-one	0.46
3α,11β-Dihydroxy-5α-androstan-17-one	0.52
3α,11β-Dihydroxy-5β-androstan-17-one	0.54
3β,16α-Dihydroxy-5β-androstan-17-one	0.55
5β-Pregnane-3α, 20α-diol	0.57
5β-Pregnane-3α, 17α, 20α-triol	0.59
3b, 16b-Dihydroxy-5-androstan-16-one	0.62
5-Androstene-3β, 16α, 17β-triol	0.63
5-Pregnene-3β, 17α, 20α-triol	0.73
3α, 17α, 21α-Trihydroxy-5β-pregnane-11,20-dione	0.73
3α, 21α-Dihydroxy-5β-pregnane-11,20-dione	0.74
3α, 11β, 17α, 21α-Tetrahydroxy-5β-pregnan-20-one	0.79
3α, 11β, 17α, 21α-Tetrahydroxy-5α-pregnan-20-one	0.80
3α, 17α, 20α, 21α-Tetrahydroxy-5β-pregnan-11-one	0.81
5β-Pregnane-3α, 11β, 17α, 20α, 21α-pentol	0.84
3α, 17α, 20β, 21α-Tetrahydroxy-5β-pregnan-11-one	0.84
5β-Pregnane-3α, 11β, 17a, 20α, 21α, pentol	0.88

to 290°C (1°C/min). Injector and detector temperatures are both 300°C. GCMS analyses are carried out with a HP 5995B quadrupole gas chromatographic–mass spectrometer equipped with a 12-m BP-1 column. The steroids are identified by their relative retention times to stigmasterol, by the ion chromatograms, and by complete mass spectra when compared to authentic standards.

14.9.2.3 Quantification of Steroids

The retention times of neutral steroids from male urine samples relative to stigmasterol are shown in Table 14.13.

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CHAPTER FIFTEEN

Environmental Applications of Gas Chromatography

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15.1 INTRODUCTION

The industrial revolution and growth of America through the nineteenth century created a great deal of prosperity in America, but it was also accompanied by a number of far-reaching environmental crises that became public and disturbed the awakening social conscience of the 1960s. Water that was once thought to be an endless resource was unsafe to drink or, in many cases, even for swimming. Many of the streams and waterways that once supported fish and wildlife were desolate. The land that was fertile and grew the amber waves of grain was marked with festering pockets of noxious, hazardous chemicals that killed and mutated the life around them. Concern for the environment and fear of the pollution that was threatening both human health and existence, created strong sentiments among Americans that it was time to address the issue of pollution. Legislators were pressured by public opinion to pass laws to regulate and monitor the chemicals being released into the environment. Concurrently the development of analytical instrumentation enabled the detection of these pollutants at trace levels. The technique of gas–liquid chromatography developed by James and Martin in 1952 became a primary tool in environmental analysis (1).

15.1.1 Historical Perspective

Environmental testing arose out of the concern for pollution in the environment. In the early 1970s Lake Erie was grossly polluted with chemicals and was dying. The Potomac River was clogged with green algae blooms that were a nuisance and threatened public health. In 1972 only a third of the nation's waters were safe for fishing and swimming (2). These and similar situations prompted the passage of The Clean Water Act in 1972 and as result, pollution control programs were established that monitored what chemicals were discharged into our streams and waters. Since its passage, considerable improvements in water quality have been made.

From the late 1960s to the 1970s, homeowners in the community in Love Canal, New York suffered a number of well-publicized health problems, such as miscarriages, chronic infections, chemical burns, internal disorders, and genetic mutations. These maladies were traced to 20,000 tons of hazardous waste buried by Hooker Chemical and Plastics Company from 1947 to 1954. President Jimmy Carter declared the town a disaster area in 1978. In 1980 the neighborhood was evacuated and in the same year, the Superfund [the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)] was created. This program was created to clean up toxic waste sites using a fund collected from chemical manufacturers and oil producers (3).

The use of DDT became widespread after World War II. A total of approximately 1.35×10^9 tons was used in over 30 years of use until it was banned in 1972. The pesticide was blamed for the decline in Raptor populations. The DDT, which bioaccumulated in the birds caused their eggshells to be thin and the eggs cracked during normal incubation. Rachel Carson, an author and government scientist wrote a famous book *Silent Spring*, which was published in 1962 (4). The book described in detail how DDT enters the food chain and accumulates in fatty tissue of animals and humans. Carson explained how the presence of these toxins causes cancer and genetic damage. The book had much effect on public opinion. In 1970 the United States Environmental Protection Agency (USEPA) was created by executive decree and in 1972 DDT was the first pesticide that it banned (5)

Although the examples cited above created the public opinion and the legislation that was required to establish the regulations, the regulations without technical capabilities were empty. Prior to 1970 only about 100 organic compounds had been identified in water. Most estimates of organic pollution came from determining total organic carbon (TOC) or biochemical oxygen demand (BOD). Finnigan Corporation produced the first stand-alone gas chromatograph and quadrupole mass spectrometer system in 1968 and a year later introduced an integrated computer data system to operate it. By 1975 nearly 1500 organic pollutants were identified in all types of water (6). The USEPA purchased six GCMS systems in 1971 and subsequently used them to develop the early GCMS methods, which were published in the *Federal Register* in 1979. Hewlett-Packard introduced the fused silica capillary column in 1979. Because of these and other advancements in technology, the environmental testing industry flourished through the 1980s and by 1991 had reached its zenith and its value was estimated

between 1.2 and 1.4 billion dollars. Because of market competition, however, it dropped to about 1 billion dollars in 1998 (3).

15.1.2 Role of Gas Chromatography in Environmental Analysis

Gas chromatography plays a central role for the determination of many organic compounds that are of environmental concern. Although it is a mature or classical technique, it has been enhanced since the early 1950s with new sample introduction techniques, columns, and detectors. These additions have made it a very valuable and versatile tool for use in the analytical methods that are required to quickly and reliably detect trace levels of numerous organic contaminants in environmental samples. When coupled with the purge and trap technique, long lists of volatile organic compounds can be determined in soil and water samples. The nitrogen phosphorus detector and electron capture detector have made the detection of pesticides at part per billion concentrations routine. The coupling of mass spectrometry with gas chromatography has provided another dimension to the data that allows for qualitative confirmation of target analytes and the tentative identification of unknown organic compounds.

Many of the gas chromatographic methods used in environmental analysis are for regulatory purposes; therefore, it is necessary to monitor large numbers and a variety of compounds with minimal effort and expense. The compounds that are present in environmental samples are often unknown unless historical records are available for a set of samples. A major theme of environmental analysis is to look for or monitor compounds in samples above a certain concentration. Gas chromatography has the unique advantage of being able to separate many compounds with a single injection and offers a huge advantage in this process. Gas chromatography has been at the center of the USEPA's strategy for monitoring organic compounds in the environment since the early to mid-1970s. It also has had a great deal of influence on the compounds that are regulated and their regulatory limit over this same time period. Improvements, such as the change from packed to capillary columns, have been incorporated, but changes to USEPA methods have been slow because of the bureaucracy and fears of adversely affecting method performance. Compromises or sacrifices have been made in many of the methods to incorporate large numbers of compounds. Optimization of methods for a few analytes is not possible with this strategy. Gas chromatography (combined with mass spectrometry) has made the separation of compound lists over 100 possible with a single injection, but recoveries of organic analytes are often far from quantitative. Improvements in instrument sensitivities have made part per trillion detection limits common.

This chapter presents the applications of gas chromatography in environmental analysis. Most of the applications that are present are established and widely practiced by environmental laboratories. The discussion is by no means a review of the current scientific literature, although some more recent developments will be sited. Other sources are available if a current review of the literature is required (7–10). Because of the large volume of information that surrounds the use of gas chromatography in environmental analysis, only selected topics

and applications that are most relevant to environmental applications of gas chromatography are presented. Topics are organized by sample preparation techniques and methods of analysis. The early part of the chapter mainly discusses the analysis of water and soil samples. The analysis of environmental air samples, which requires totally different methods of sample handling and sample introduction, is discussed near the end of the chapter.

15.2 GOVERNMENT REGULATION IN ENVIRONMENTAL ANALYSIS

Most of the environmental cleanup and testing that has occurred since the early 1970s have been the result of government regulation. Industries have been forced by law to show that they are not polluting the environment by improperly discharging toxic chemicals or to clean up existing hazardous-waste sites. Government agencies have been created to enforce these laws and to see that environment does not become polluted. The following sections will briefly discuss some of the major legislation and agencies that were created to protect the environment.

15.2.1 Major Federal Legislation

Most of the federal legislation to control pollution was enacted after 1970. This legislation identified priority pollutants and hazardous chemicals that were a threat to the environment. Allowable limits for the discharge of chemicals into the environment were also established. To measure these chemicals, standardized analytical methods and detection limits were published. Some of the major federal environmental legislation is listed chronologically in Table 15.1. Most of the analytical methods that are used by the environmental laboratory originate from the legislation listed in Table 15.1. When a laboratory is required to determine a compound in an environmental sample, these methods are often referenced. The method that is chosen will depend on the class of the organic compound, the type of sample, and the detection limit that is required.

The 600 series methods were published in *The Code of Federal Regulations* as a result of the Clean Water Act and its amendment for use with the National Discharge Elimination System (NPDES) permitting process and enforcement (12). Methods for both inorganic and organic analysis are listed. The methods were last updated in 1984 and still reference packed columns although the CFR is published yearly. Gas chromatographic methods for the determination of volatile and semivolatile volatile compounds, pesticides, and poly(chlorinated biphenyl)s (PCBs) using a variety of detectors are listed. Whenever a laboratory is required to analyze NPDES samples the method guidelines and quality control requirements must be met although specific technical changes to the methods (e.g., capillary column substituted for a packed column) are allowed.

The 800 series methods were published in *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, 3rd edition, USEPA Publication

TABLE 15.1 Major Federal Legislation Regulating the Environment

Environment Law	Directive
Water Pollution Act 1942	Directed Public Health Service to provide grants for industrial waste studies and construction of treatment works
Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) 1947; amended by Federal Environmental Pesticide Control Act 1972; amendments in 1975, 1978, 1980, and 1988	Restricted sale and distribution of pesticides; all pesticides must be registered with USEPA and be properly labeled; pesticide may have no adverse effect on the environment when properly used
Clean Air Act 1970; amended in 1977	Each state has responsibility to achieve set National Ambient Air Quality Standards (NAAQSs) for major air pollutants; USEPA required to set national emission standards for hazardous air pollutants
Clean Water Act 1972; amended in 1987	Established National Discharge Elimination System (NPDES); authorized USEPA to grant permits to discharge pollutants into public waterways provided standards are met
Safe Drinking Water Act 1974; modified in 1986	Effort to protect nation's drinking water supply; required USEPA to establish maximum contaminant limit goals (MCLGs) for contaminants
Resource Conservation Recovery Act 1976	Defined hazardous waste and defined rules treatment, transport, storage, and disposal
Toxic Substances Act 1976	Required industry to test certain substances to determine if they posed a threat to health or the environment and authorized USEPA to limit or ban hazardous materials
Comprehensive Environmental Response, Compensation and Liability Act (CERCLA or Superfund) 1980; amended in 1986	Created a tax on crude oil and commercial chemicals to support the cleanup of abandoned or uncontrolled hazardous-waste sites

Source: Adapted from References 6 and 11.

SW-846 (13). These methods were published as a part of RCRA and include inorganic and organic analysis. Gas chromatographic methods for the determination of volatile and semivolatile compounds, pesticides, and PCBs using a variety of detectors are listed. These methods were last updated in 1996 and make use of the capillary column. Environmental laboratories use these methods very heavily for regulatory purposes.

The 500 series methods were published in *Methods for the Determination of Organic Compounds in Drinking Water* (14). These methods were published to support the Safe Drinking Water Act of 1974 and focus on determining low concentrations of inorganic and organic analytes in drinking waters. The 500 series methods last underwent a major revision in 1991, although specific methods have been updated since. These methods are written to stand alone and are referenced in this chapter individually.

As part of CERCLA or the Superfund, a set of methods was published in 1990 for contract laboratories performing inorganic and organic analysis (15). In the *Statement of Work for Organic Analysis*, methods for the determination of volatile and semivolatile compounds in water and soil using gas chromatography and mass spectrometry (GCMS) were published. A method for determining organochlorine pesticides and PCBs using gas chromatography and the electron-capture detector (GCECD) in soils and water was also given. Strict guidance on sample handling, contract required quantitation limits, and quality control requirements were presented in these documents. Even the formatting and reporting of both paper and disk-deliverable data was outlined. This document has been revised several times and the last updated was in 1999 (16).

15.2.2 Government Agencies

The United States Environmental Protection Agency (USEPA or EPA) was established by executive decree in 1970. The USEPA was created to provide the government with a centralized focus and knowledge necessary to clean up pollution and address the environmental problems that existed within the country. The USEPA interprets environmental laws passed by congress and develops operating procedures to meet the requirements of those laws. Although the USEPA is the most widely recognized federal agency that regulates the environment, other federal agencies play significant roles. Some of these federal agencies are highlighted in Table 15.2.

15.2.3 Role of the States

Protection of the environment occurs at all levels of government. Most federal environmental laws give the states the “right of first refusal” to regulate environmental methods within their borders. Failure to exercise that right results in federal regulation (3). The states have their own departments for protecting and monitoring the environment. Many of the states have their own laboratories and analytical methods that must be followed for the analysis of samples taken within the boundaries of the state. Often the states defer to the USEPA methods listed in one the references discussed in Section 15.2.1 or to an adaptation thereof. Sometimes states will copy the guidelines and methods of another state and adopt them as their own.

TABLE 15.2 Federal Agencies that Administer to the Environment

Federal Agency	Function
Council on Environmental Quality	Advises president on environmental issues and prepares annual report to Congress
National Oceanic and Atmospheric Administration	Studies environmental data to address global problems
U.S. Army Corps of Engineers	Administers dredge and fill permitting of waterways under the Clean Water Act
Department of Energy (DOE)	Ensures that the Energy Department programs comply with environmental standards
Nuclear Regulatory Commission	Regulates commercial use of nuclear energy to protect the public and environment
Department of Transportation-Environmental Division	Develops environmental policy and monitors implementation of environmental laws
Bureau of Land Management Department of the Interior (DOI)	Administers public land for fishing, wildlife management, recreation, grazing, timbering, industrial development, watershed protection, and mineral development
Bureau of Mines (DOI)	Develops technology essential for supplying the mineral needs of the country and while also protecting the environment
U.S. Geological Survey (DOI)	Maps and reports on the physical features of the United States and its, mineral, fuel, and water resources
Bureau of Reclamation (DOI)	Administers federal program for development of water resources
Office of Surface Mining (DOI)	Administers programs to protect the environment from strip mining operations; promotes land reclamation from abandoned mines
Occupational Safety and Health Administration	Develops occupational safety and health standards and regulations

Source: Adapted from References 3 and 17.

15.3 ENVIRONMENTAL SAMPLES

Environmental samples can be extremely complex and come from a great variety of sources. Each sample requires a sampling and storage strategy. Analytical methods need to include procedures for sample preparation, extraction, and, if necessary, cleanup prior to gas chromatographic analysis. Complex and dirty samples require more difficult sample preparation techniques. Samples can be solids, liquids, gases, or mixtures of these phases. For example, a sample pulled

from a drum of hazardous waste may have layer of sludge on the bottom, an aqueous layer in the middle, and an upper layer of some unknown organic solvent. To obtain an accurate chromatographic analysis of the sample, each layer may require a different strategy for extraction and clean up. Fortunately, not every environmental sample is so complex. Water samples and soil samples comprise the majority of environmental samples that undergo gas chromatographic analysis. Aqueous samples can include surface water, groundwater taken from an aquifer, leachate from a landfill or toxic-waste site, industrial wastewater, treated sewage effluent, or even a finished drinking water that has been disinfected with chlorine or bromine. Waters may have different chemical and physical properties that may alter their behavior during extraction and cleanup. Waters may have different amounts of suspended and dissolved solids. They can be of varied pH and contain different levels of total organic carbon (TOC). Natural waters contain fulvic acid from humus and decaying vegetation that can produce a brownish color and create a high TOC. Groundwater can often contain high levels of calcium carbonate that produces an alkaline and highly buffered pH. Other environmental, liquid samples can include unknown, nonaqueous solvents from drums or storage tanks that require characterization or identification by gas chromatography.

Soil samples may be taken near the surface or from under the surface using drilling equipment. Sandy soils that have larger soil particles are much easier to extract than clay soils and usually do not contain high amounts organic matter that can interfere with a gas chromatographic analysis. Organic interferences, coextracted from loams and soils that have large amounts of organic humus, can obscure analyte peaks, alter detector response, or create false positives on a gas chromatogram. For simplicity, sediments will be defined as soil that has been displaced and deposited by a force of nature, usually moving water. Marine sediments are an analytical challenge because of high levels of sulfur that are often present. Recoveries of analytes are often lower than normal from clay soils, which contain very small particles and have a large surface area. This is especially true for polar analytes that bind almost irreversibly with silanol groups on the soil surface producing low recoveries (18). Other Environmental solid samples include sludge from industrial processes and municipal sewage treatment facilities, and flyash from waste incinerators and coal-burning power plants.

Air samples can be collected from atmospheric air or from gases that are emitted from industrial sources. These air samples might contain high levels of carbon dioxide, carbon monoxide, nitrogen dioxide, and sulfur dioxide. Air can be collected outside, for instance, around the perimeter of a toxic waste site or inside as a fugitive emission from an industrial process. In the latter case the line of demarcation between environmental analysis and industrial hygiene is not so clear. Sometimes the air is collected and held in a gastight container and in other cases analytes are extracted from the air during the sampling process by passing it through solid-phase sorbents or liquid impingers.

15.3.1 Collection of Samples

If a sample has not been collected properly, the quality of the data will be diminished. When environmental samples are collected, several requirements must be met. The sample must be a representative sample of the location or area from which it is taken. Environmental engineers and hydrogeologists usually spend a great deal of effort in mapping out sampling sites to optimize the location of drill borings or monitoring wells to obtain an accurate assessment of a contaminated area. Sampling strategies and determining the location of monitoring wells are beyond the scope of this chapter. Soil, water, and air samples can be collected as a grab sample or as a composite sample. Grab samples are taken at one location at one particular time. Samples taken from different locations at a site can be combined and mixed to form a composite sample. Soils are often sampled at different locations at the sampling site and mixed proportionally to produce a single composite sample. Time composite samples are collected over a given period of time (e.g., 24 h) by an automatic sampling device. Time composite samples are often collected at a discharge point when the composition of the sampling stream can vary over time. A time composite sample might be taken over 24 h to obtain a representative sample of an industrial discharge into a sewer line or a stream so that times when the plant is operating at full production and slower production times are included in the composite sample. Time composite samples are often required for air sampling because of the transient nature of air.

The sample must be of sufficient quantity so that the detection limit of the method can be obtained. The sample must be collected in a timely fashion and preserved so that the analytes of interest remain intact until the sample can be transported and analyzed at the laboratory. Mobile laboratories placed on the sampling site or portable field chromatographs have been used to overcome the delay in shipping samples to the laboratory. However, most samples can be shipped overnight to laboratories in the continental US using commercial air carriers.

15.3.2 Handling and Storage of Samples

Many methods supplied by the USEPA outline the way a sample is to be preserved and stored prior to analysis. Soil and water samples are usually kept cold (4°C) from the time of collection to analysis. Samples are often shipped in insulated coolers containing ice packs or dry ice. Certain organic compounds breakdown when exposed to ultraviolet light. Samples that are suspected of containing these compounds should be collected in amber-colored bottles and stored in the dark. If a sample has been collected from a public water supply that has been chlorinated, sodium thiosulfate should be added at the time of collection to remove any free chlorine that may be present. Often times water samples are pH-adjusted to less than 2 using a strong acid such as hydrochloric or sulfuric acid to retard microbial breakdown of organic analytes. The length of time

from when a sample is collected until it can be analyzed is referred to as the holding time. The USEPA and other regulatory agencies often set limits on the holding time for various classes of organic compounds. Many weekends have been ruined for the environmental analytical chemist because of holding times. Table 15.3 presents information on sample collection, preservation, and holding times for a number of relevant organic compounds. Sample preservation techniques and holding times can vary from those listed in Table 15.3 if they are taken from a different regulatory method or specially defined by a project.

Most environmental laboratories supply their clients with sample kits that are required for a specific project. The laboratory will send coolers containing the necessary bottles containing preservatives to a sampling site. The bottles are filled at the site during sampling and packed in ice or dry ice and returned to the laboratory using an overnight carrier. For legal reasons and to meet certain regulations a chain-of-custody form will often accompany the samples. Each time a sample or a portion of the sample changes hands from the time the sample is collected until it has been analyzed, the data have been reported, and the sample has been discarded, a documented record is kept. A detailed history of the sample from cradle to grave can be reconstructed if necessary from the chain of custody and other laboratory records.

TABLE 15.3 Sample Handing and Preservation of Environmental Samples Requiring Organic Analysis

Compound Class	Sample Matrix	Recommended Collection Vessel	Number of Vessels	Preservative	Holding Time (days) at 4°C
Volatile organic compounds	Water	40-mL glass vial with Teflon-lined septum	2	HCl to pH < 2; no headspace	14
	Soil	100-mL wide-mouthed jar with Teflon-lined lid	1	None	14
Semivolatile organic compounds	Water	1-L amber glass bottle with Teflon-lined lid	2	None	7 40 after extraction
	Soil	500-mL wide-mouthed jar with Teflon-lined lid	1	None	7 40 after extraction
Pesticides and PCBs	Water	1-L amber glass bottle with Teflon-lined lid	2	None	7 40 after extraction
	Soil	500-mL wide-mouthed jar with Teflon-lined lid	1	None	7 40 after extraction

Source: Adapted from USEPA SW846, Reference 13.

During the collection, shipment, and storage of samples, contamination can occur. In the field a sample may be contaminated from the sampling apparatus or it can be contaminated from other samples. Volatile organic compounds present in the air can diffuse through the lids of containers and contaminate samples during shipment and storage. For these reasons special blanks (besides the method blank that is generated in the laboratory and carried through the analytical procedure) are often associated within a set of samples. A trip blank consisting of laboratory reagent water sealed in a 40-mL vial will often be shipped to the sampling site with the sample bottles. When the samples are collected the trip blank will be sent back, unopened, for VOC analysis with the samples. A field blank is often required for each compound that is analyzed in the laboratory. When the sample is collected in the field, the field blank is also collected. Usually reagent water that is used to rinse or has passed through the sampling device is collected and sent to the laboratory in similar sampling bottles for analysis.

15.4 CLASSES OF COMPOUNDS DETERMINED BY GAS CHROMATOGRAPHY

Organic compounds that are known to be of environmental concern and can be separated by gas chromatography are included in this discussion. Many of these compound are considered priority pollutants and are regulated in some manner. The strategy of the USEPA has been to group compounds with similar chemical properties and structures together and to determine them using a standardized analytical method. Gas chromatography has been conducive to this philosophy since it allows for the separation of many compounds using a single injection. Analytical standards must be made and maintained for a large number of compounds. For example in the analysis of semivolatile organic compounds (SVOCs) using USEPA method 8270 over 100 compounds can be on the compound list (13). The GCMS must be calibrated for each compound and meet the quality control (QC) requirements of the method. The gas chromatograph must be maintained and conditions optimized to successfully separate and quantify all these compounds. It takes a very skilled analyst to perform these methods and meet all the requirements. Small changes in instrument conditions may preclude the successful analysis of a number of compounds on the list of analytes. Many gas chromatographic methods that are employed are not optimized for a specific compound but are optimized to encompass larger lists of compounds. The common classes of environmental compounds are discussed below.

15.4.1 Volatile Organic Compounds

Volatile organic compounds (VOCs), generally have a boiling point less than 200°C and a vapor pressure greater than 0.1 Torr at 25°C and atmospheric pressure. Usually a gas-phase extraction by static or dynamic headspace sampling is used to separate VOCs from an aqueous or solid samples for introduction into

the gas chromatograph. Included in this category are light aliphatic hydrocarbons and aromatic hydrocarbons from petroleum sources that have contaminated the environment. Environmental regulators and laboratories frequently refer to these gasoline-range organics by the acronym GRO. Aromatic compounds that are a subset of GRO are benzene, toluene, ethylbenzene, and the xylene isomers. These compounds are collectively known by the industry as BTEX. The upper range for purgeable hydrocarbon is usually about C10, with naphthalene often as the last peak on a gas chromatogram for this group of compounds. Halocarbons, short-chained hydrocarbons (up to C4) containing fluorine, chlorine, and bromine, are another subset of VOCs. Many of these compounds, such as trichloroethane were used as industrial cleaning solvents and improperly dumped onto the ground. The common freons, such as Freon 113, trifluorotrichloroethane, were used as refrigerants and degreasers. Freon 11, difluorodichloromethane, is an extremely volatile compounds and a gas at room temperature. The light gases, such as Freon 11, present an analytical challenge because they are difficult to focus on the chromatographic column without the use of cryogenic cooling. The halocarbons are somewhat water-soluble and migrate rapidly through the soil. The carbon–halogen bond also makes them somewhat resistance to microbial breakdown. When they reach an underground aquifer their plume of pollution migrates very slowly and remains for decades. Because of the halogen that is present in these compounds, the electrolytic conductivity detector (ELCD) or Hall detector is often used in the methods for their detection.

Short-chain alcohols, ethers, acetates, and ketones round out the list of VOCs. These compounds are difficult to purge from water because they are very soluble in water. Methyl tertiary butyl ether (MTBE), added to gasoline in place of lead as a no-knock agent, has been found in a growing number of aquifers throughout North America and has become a primary environmental concern. Similarly, tertiary butyl alcohol (TBA), another oxygenate found in gasoline, has also been a growing concern. Common ketones include acetone, 2-butanone (MEK), 4-methyl-2-pentanone (MIBK), and 2-hexanone. Some common alcohols considered VOCs are 1-propanol, 2-propanol (isopropanol), and n-butanol. Vinyl and ethyl acetate are also common VOCs belonging to the acetate family.

15.4.2 Semivolatile Organic Compounds

The category of environmental compounds known as *semivolatile organic compounds* (SVOCs) are also referred to as *extractables*, because an extraction technique such as a liquid–liquid extraction or a Soxhlet extraction must be used to separate these compound from water and soil. Compounds that are protonated at a low pH, have a neutral charge, and partition into the organic phase during a liquid–liquid extraction are referred to as *acid extractables*. A major class of compounds that are acid extractables and of environmental concern are the phenols. The phenols contain a benzene ring with a hydroxyl group attached and are substituted at various positions with chloro, methyl, and nitro groups. The polar hydroxyl group on the phenols can make these compounds very difficult to separate by gas chromatography. Peak tailing is caused by interaction

of the hydroxyl group with active sites in the gas chromatograph. Compounds that have a neutral charge at elevated pH are referred to as *base extractables*. These include the substituted analines and amines. The anilines contain a benzene ring with an amine group attached, and like the phenols are substituted with chloro, methyl, and nitro groups. Other examples of amines include diphenylamine, the isomers of diaminotoluene, benzidine, and 3,3′-dichlorobenzidine. Some of the nitrosamines, cancer-causing agents found in tobacco smoke, are also listed as priority pollutants. Nine of these compounds are listed in USEPA SW846 Method 8270 (13). These compounds contain the nitroso group (–N–N=O) and are byproducts from the manufacture of rubber.

A large number of the SVOCs have no charge in aqueous solution regardless of the pH and are referred to as neutral compounds. Included in this category are the polynuclear aromatic hydrocarbons (PAHs). The PAHs consist of two or more aromatic rings that share a pair of carbon atoms. Many of the PAHs are suspected carcinogens and originate from petroleum products and combustion processes. They range in size from naphthalene (also included with the VOCs) consisting of two aromatic rings to benzo(g,h,i)perylene, which has six aromatic rings and a molecular weight of 276 amu. More than likely this compound will be last of the SVOCs to elute from a gas chromatographic column. Figure 15.1 shows the structures of some common PAHs.

The phthalate esters are used to manufacture plastics to impart flexibility and toughness (plasticizers). As a result, they are present in the environment and are suspected carcinogens. These compounds are esters of phthalic acid. Seventeen of these compounds are listed in the USEPA SW846 gas chromatographic method, 8261 (13). Bis(2-ethylhexyl)phthalate is perhaps the most common of the phthalate esters and has been the bane of many environmental laboratories, because of its ability to find its way into the most well-prepared method blank.

A significant number of chlorinated haloethers, chlorinated hydrocarbons, and chlorinated benzenes are SVOCs. Examples include bis(2-chloroisopropylether), 4-chlorophenylphenylether, hexachloroethane, isomers of dichlorobenzene, isomers of trichlorobenze, isomers of tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene, to name only a few. A number of nitroaromatic SVOCs are considered environmentally significant. Remedial action at an old military installation might require the determination of isomers of nitrobenzene, dinitrobenzene, dinitrotoluene, and trinitrotoluene (TNT) in soil and water samples, because they are indicative of explosives.

15.4.3 Pesticides and Polychlorinated Biphenyls (PCBs)

Although semivolatile in nature, the pesticides and PCBs are given their own category in environmental analysis. Because of the toxicity of these compounds, lower detection limits are often required. For pesticides and PCBs two primary gas chromatographic detectors are used. The electron-capture detector (ECD) enables subpart-per-billion detection for organochlorine pesticides and the PCB congeners. The nitrogen phosphorus detector is used primarily for the organophosphorus and

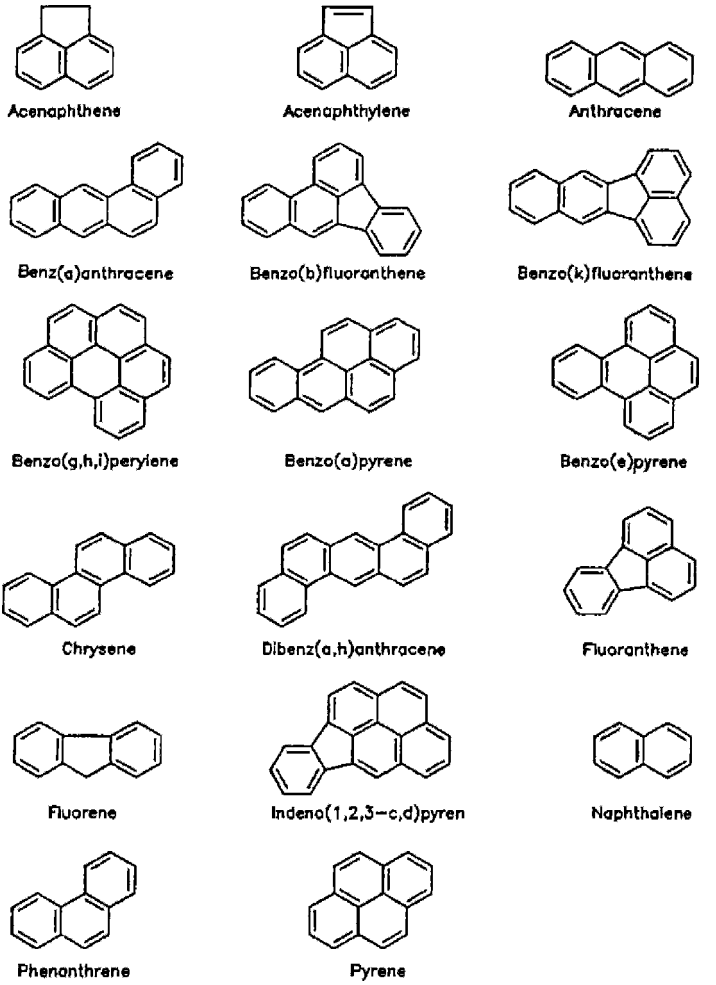


FIGURE 15.1 Chemical structures of common polynuclear aromatic hydrocarbons (PAHs) (taken from Reference 121).

nitrogen-containing pesticides. The organochlorine pesticides are more persistent in the environment than are the organophosphorus pesticides, because they are less susceptible to oxidation, hydrolysis, and microbial decomposition. They are hydrophobic and less polar than the organophosphorus pesticides and move more slowly through the environment. As they are composed of mostly carbon, hydrogen, and chlorine, they have hydrocarbon characteristics. They are lipophilic and can bioaccumulate in the food chain, having been found in the fatty tissue of many animals, including humans. The best known of the organochlorine pesticides is probably DDT (1,1,1-trichloro-2, 2-bis (4-chlorophenyl). The structure of DDT is shown in Figure 15.2. Since 1973 it has been banned in the United

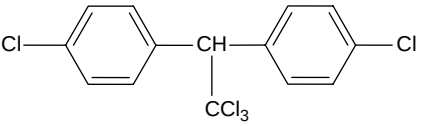


FIGURE 15.2 Chemical structure of DDT (1,1,1-trichloro-2, 2-bis (4-chlorophenyl).

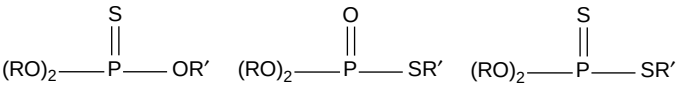
States and has been blamed for the decline in the bald eagle and peregrine falcon populations. The pesticide can remain in the soil for years and forms its metabolites, DDD and DDE, which are also monitored (2,19).

The organophosphorus pesticides are more polar and more soluble in water than the chlorinated pesticides. For this reason they are more mobile in the environment than the organochlorine pesticides. Although more acutely toxic than organochlorine pesticides, they undergo oxidation, hydrolysis, and microbial decomposition and not likely to persistent in the environment as long. Approximately 50 of these compounds are monitored using the USEPA SW846 Method 8141 (13). The organophosphorus pesticides fall into three categories: phosphorothionates, phosphorothiolates, and phosphorodithioates. These categories are shown in Figure 15.3. Parathion and diazinon are examples of phosphorothionates, which is the most common category of organophosphorus pesticide. Demeton is a well-known phosphorothiolate and malathion is an example of a phosphorodithioate (20).

The triazine herbicides, atrazine and simazine, should be mentioned here because they also respond on the nitrogen–phosphorus detector. These herbicides contain the triazine ring, which is a six-member, conjugated ring containing alternating carbon and nitrogen atoms.

Although banned since 1978, PCBs continue to remain in the environment and are the focus of many environmental studies and remedial actions. The structure consists of biphenyl that can have up to 10 chlorine atoms attached. The generalized structure of a PCB is shown in Figure 15.4. Depending on the number and location of the chlorine atoms attached to the biphenyl ring, a total of 209 different congeners can exist. Each of the congeners has been assigned an IUPAC number (sometimes called a *Ballschmitter* and *Zell* or BZ number), following the IUPAC rules of substitution in biphenyls (21).

Polychlorinated biphenyls were manufactured as technical mixtures known as *Aroclors* and were used to insulate electrical transformers. The common practice



phosphorothionates phosphorothiolates phosphorodithioates

FIGURE 15.3 Categories of organophosphorus pesticides (adapted from Reference 20.)

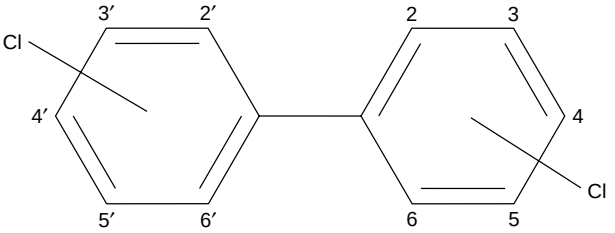


FIGURE 15.4 General chemical structure of a Polychlorinated Biphenyl.

has been to report the PCBs as these technical mixtures. Common Aroclor mixtures include 1016, 1232, 1221, 1248, 1254, and 1260. The name Aroclor 1260 implies that 12 carbon atoms are present and that 60% of the material is chlorine. Aroclor 1260 is made up of a mixture of congeners that produce a distinctive pattern when injected into a gas chromatograph equipped with an ECD.

Polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) can be introduced into the environment by combustion processes and are of major concern surrounding municipal and hazardous-waste incinerators. The general structure of the dibenzodioxin and the dibenzofuran are shown below in Figure 15.5. The most publicized and the most toxic of the dioxin isomers is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This isomer was found in the herbicide, Agent Orange, used as a defoliant during the Vietnam War. Agent Orange was blamed for a number of health problems and sickness suffered by Vietnam Veterans.

15.4.4 Miscellaneous Compounds of Environmental Concern

Certain organic compounds are too polar and nonvolatile for gas chromatographic analysis. The chlorinated acid herbicides fall into this category. Included in this

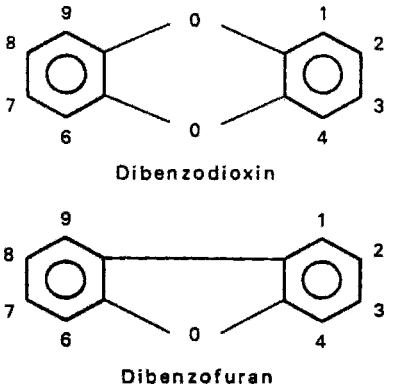


FIGURE 15.5 General chemical structures of dibenzodioxin and dibenzofuran; structures chlorinated at any numbered position (taken from Reference 13.)

list are the common phenoxy acid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid), 2,4-DB (2,4-dichlorophenoxybutanoic acid), 2,4,5-TP (2,4,5-trichloropropanoic acid), and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Diazomethane is used to derivatize these compounds to form the more volatile methylester for gas chromatographic analysis. Disinfectant byproducts are caused when chlorine and bromine are added to disinfect drinking water and react with the natural organic material present in the water. The short-chained halogenated organic acids that can be formed are suspected carcinogens. Trichloroacetic acid, dichloroacetic acid, dibromoacetic acid, and bromochloroacetic acid are examples of disinfectant byproducts and also are derivatized to their methylesters for gas chromatographic separation.

A number of organometallic compounds are of environmental concern and are amenable to gas chromatography. Highly toxic methyl and ethyl mercury can form when mercury introduced into the environment reacts with organic carbon (22). Alkylated species of tin are of concern in harbors and shipyards because of the use of biocides used to protect ships from mussels (23). Although no longer added to gasoline, tetraethyl lead can be of environmental concern and be determined by gas chromatography (24). Alkylated species of selenium can also be determined using gas chromatography (25).

15.5 HEADSPACE SAMPLING OF VOLATILE ORGANIC COMPOUNDS IN ENVIRONMENTAL ANALYSIS

When gas chromatography is applied to environmental samples to determine VOCs, headspace sampling is almost always used. Headspace sampling or the vapor equilibrium technique is a gas–liquid extraction in which the analytes of interest are partitioned into the gas phase from the sample matrix for introduction into the gas chromatograph. Because the mobile phase in gas chromatography is a gas such as nitrogen, helium, or hydrogen, this technique is very amenable to gas chromatography. The high vapor pressure of volatile organic compounds also favors their partitioning into the gas phase. Because nonvolatile and less volatile compounds in the sample remain behind during headspace sampling, the separation is cleaner and the chance for contamination of the gas chromatographic inlet, column, or detector is much less. It is naturally a much cleaner technique than liquid–liquid extraction, which can introduce heavier coextracted organic compounds into the gas chromatograph. Headspace sampling can be segregated into the simple but elegant static technique or the more commonly used technique in environmental analysis, dynamic headspace sampling.

15.5.1 Static Headspace Sampling

When a gas phase and a liquid phase are present in a closed system, an analyte will partition itself between the gas phase and the liquid phase until equilibrium

is reached. The distribution coefficient K may be defined as

$$K = \frac{C_l}{C_g} \tag{15.1}$$

where C_l is the concentration of the analyte in the liquid phase and C_g is the concentration of the analyte in the gas phase. Generally in environmental analysis the liquid phase is usually aqueous. Volatile compounds that have a low solubility in water and have a high vapor pressure will have a low K value and readily partition into the headspace. Headspace gas chromatography (HSGC) will be more sensitive in determining these compounds. Aromatics (e.g. benzene, $K = 4$), hydrocarbons, and halocarbons fall into this category. Compounds that are more soluble in water and have a low vapor pressure will have a much higher K value and favor the aqueous phase. Polar compounds such as short-chained alcohols (e.g., ethanol, $K = 4000$), ketones, amines, and aldehydes are included in this group and are more difficult to detect using HSGC (26).

Static headspace is a simple technique and requires less equipment than dynamic headspace sampling. A gastight syringe, glass vials fitted with septa, and a thermostatted device, such as a temperature bath, are the only other equipment requirements besides the gas chromatograph. Sample preparation for HSGC is very simple. An aliquot of an aqueous sample is measured into a headspace vial and usually a salt is added to saturate the solution and to create sample solutions that have uniform ionic strength. Commonly used salts are sodium chloride, sodium sulfate, and potassium chloride. The addition of the salt also lowers the solubility of the VOCs in the water and helps partition them into headspace. Similarly, soils are weighed into vials and a saturated salt solution is added. The vial is then sealed with a septum and incubated at an elevated constant temperature. After equilibration, an aliquot of the headspace is withdrawn from the vial and injected into the gas chromatograph for separation. Heating the vial shifts the equilibrium toward the headspace and increases C_g. Temperatures close to the boiling point of water (ca. $>90^{\circ}$C) increase the amount of water vapor in the headspace, which can adversely effect the gas chromatography by overloading the stationary phase or suppressing the response of the detector. If an elevated temperature is used special precautions may be required to trap the water (27).

The phase ratio β in the vial may be defined as

$$\beta = \frac{V_g}{V_l} \tag{15.2}$$

where V_g is the volume of the gas and V_l is the volume of the liquid. If quantification is required the phase ratio has to be constant for standards and all samples. If K is large, C_g will not be influenced by the phase ratio. However if K is small, a higher β will increase C_g and a smaller β will decrease C_g. In the latter case, the concentration of the analyte in the headspace may be diminished and the sensitivity of the method lost (28).

Another important step in the method is the injection. Large injection volumes will overload the injection port and distort peak shape. Extremely volatile compounds such as the light gases (e.g., chloromethane, bromomethane, chloroethane, dichlorodifluoromethane, vinyl chloride) will limit the injection size because they are difficult to focus onto the head of the column. These compounds may appear as broad or smeared peaks on the chromatogram resulting in poor sensitivity and integration. Cryogenic cooling or focusing may be required for large injection volumes. The headspace syringe must also be warm enough during the injection to prevent heavier analytes from condensing on the barrel. Care must also be taken to ensure that the syringe needle does not get plugged when the septa are pierced. A plugged syringe needle has caused many clean headspace chromatograms.

Headspace sampling can be automated, however, and a number of automatic headspace samplers are commercially available. Some of these samplers pressurize the headspace vial with carrier gas prior to filling a sampling loop for injection. These samplers allow unattended operation, but are much more complex and require significant method optimization prior to use. A number of manufacturers are listed in Reference 29.

Although simpler and less expensive, static headspace sampling is not considered as sensitive as dynamic headspace analysis. In static headspace sampling only a portion of the headspace is sampled, while in dynamic headspace sampling the entire sample is quantitatively purged of volatile analytes. Most USEPA chromatographic methods for determining VOCs in water and soil use dynamic headspace sampling. Method 5021 is the static headspace method listed in SW846 and it is listed as a general-purpose method for soils, sediments, and solid waste (13).

Quantification using static headspace is difficult because K is seldom known for most practical applications. The problems and various approaches to this problem have been addressed (30–32). If the sample matrix is clean water, then a simple external standard method can be used. Unfortunately few environmental samples fall into that category. Aqueous samples are a simpler matrix and more homogenous than soils. However, waters with high organic content can be difficult to accurately quantify using HSGC (33). The method of standard additions may be used if the sample is homogeneous and sufficient sample is available. First the sample is analyzed using HSGC. Another headspace vial of the sample is then spiked with the analyte(s) of interest to obtain (hopefully) a response that is approximately one and a half to double the response found in the unspiked sample. Several spikings, preferably three or more, should be performed. The responses are plotted against the amount of spike that was added to obtain a linear curve. Extrapolation back to the X axis ($Y = 0$) yields the concentration in the sample. The use of multiple headspace extraction (MHE) provides another possible solution to quantification (34). The technique was introduced by McAuliffe (35) and was used adapted by Kolb to handle solid samples (36). Initially the headspace of the sample in a sealed vial is equilibrated and analyzed. A given portion of the headspace is withdrawn and replaced with an equal volume of clean gas or air. Again the sample is equilibrated and the analyte(s) of

interest is determined in the headspace. After several repetitions of the process, a plot of the natural log of the peak area versus the number of injections is made. This plot produces a linear curve that can be defined as

$$\ln A = -MN + q \tag{15.3}$$

where A = peak area (Y)
N = injection number (X)
$-M$ = slope of the line
q = the y intercept

The total area of all the analyte A_{tot} in the sample from an infinite number of equilibrations can be determined by

$$A_{\text{tot}} = \frac{A_1}{1 - e^{-M}} \tag{15.4}$$

where A_{tot} = total peak area for an analyte that would be obtained from an infinite number of equilibrations and exhaustively extract the analyte from the matrix
A_1 = area of the first injection
$-M$ = slope from Equation 15.3

The total mass of the analyte in the sample is determined from its response factor and A_{tot}.

15.5.2 Dynamic Headspace Sampling

Dynamic headspace sampling, or commonly referred to as "purge and trap," is the technique associated with most of the USEPA methods. Bellar and Lichtenberg developed the technique in 1974 (37). The USEPA SW-846 method using purge and trap for aqueous samples is 5030B (13). Because the sample is thoroughly purged and the analyte(s) are believed to be quantitatively removed from the sample, lower detection limits and better quantification are theoretically possible compared to static headspace sampling. The equipment, however, that performs the purging and trapping is more expensive and more complicated to use. Water samples are usually measured with a syringe from a vial that has been sealed and stored with no headspace. After the addition of the appropriate surrogate or internal standards, the water is injected into the purging vessel. Automated samplers are commercially available that will keep the sample vials cool, load the purge vessel, and add the internal standards and surrogate standards (29). Usually 5 mL of water is purged (13); however, USEPA Method 524 for drinking water stipulates that, if necessary, 25 milliliters of sample are to be used to obtain lower detection limits (14). The purging vessel, an example is shown in Figure 15.6, has a fritted-glass bottom that allows the purge gas, usually nitrogen or helium,

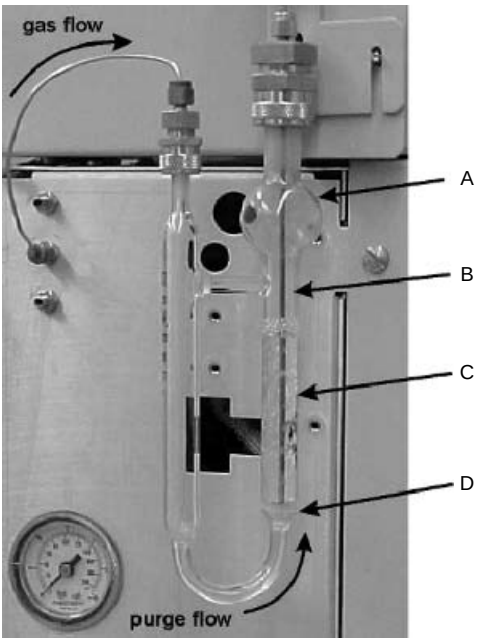


FIGURE 15.6 Picture of purge vessel used in dynamic headspace sampling: A—glass purge vessel; B—stainless-steel sample delivery tube; C—5-mL water sample being purged; D—glass frit for dispersing Helium gas bubbles.

to pass through. Fine gas bubbles are created by the glass frit that sweep through the sample and strip away the VOCs.

Generally, purge times of 10–15 min with purge gas flows of 40–50 mL/min are used. The purge vessel is often heated using a thermostatted heating jacket or an infrared light to encourage VOCs into the gas phase. Purge temperatures that are too high (greater than ~50°C) can introduce excessive water vapor into the trap and cause trapping or separation problems. Most commercial purge and trap units have a water management system immediately downstream from the trap. Essentially they are an inert heatsink that is temperature-controlled to condense and collect water vapor during purging. After purging these devices can be vented and baked at an elevated temperature to remove the excess water in preparation for the next sample.

Soil samples are usually handled in one of two ways. Often the soil is first extracted with methanol, an aliquot of the methanol is added to water, and the water is purged in the usual way. The USEPA SW846 Method 3585, *Waste Dilutions for Volatile Organics* (13), is used on these "high-concentration soils." Sometimes as a means of preserving the sample, methanol is added to the soil when it is collected in the field. The other technique, for "low-concentration soils," employs USEPA SW846 Method 5035 (13). Five grams of soil and sodium bisulfite are added to a 40-mL headspace vial in the field. At the laboratory prior

to analysis, water and the appropriate surrogate standards are added to the vial through the septum so that no VOCs from the sample can escape prior to purging. The vial is heated to 40°C and purged with a stream of helium through a sparge needle that is inserted into the bottom of the vial through the septum.

During the purging process, the purge gas flows through the trap, usually $\frac{1}{8}$-inch stainless steel tubing that is packed with a solid adsorbent or with multiple beds of adsorbent materials. By using several adsorbents, VOCs with a broad range of polarity and molecular weight can be effectively trapped. Table 15.4 lists some of the adsorbents that are commonly used in packing traps.

A commonly used trap for a wide range of VOCs contains about an 8-cm length of Tenax closest to the purge vessel followed by an 8-cm length of silicagel and an 8-cm length of activated charcoal. The Tenax adsorbs compounds that boil above approximately 35°C. Tenax has a strong affinity for polar compounds and holds more water than do most of the other adsorbents. Silicagel catches the low boilers except for very volatile light gases. These compounds are trapped by the activated charcoal. Care must be taken to ensure that any of the analytes of interest do not break through the trap. Extremely small and light compounds such as methane and ethane are not effectively trapped by most sorbents at room temperature. However, the trap can be cryogenically cooled to trap extremely volatile compounds. The use of cryogenic cooling adds extra expense to the analysis and can be a nuisance. When cryogenically cooled, the trap is also more efficient at holding water and carbon dioxide. The water can freeze and plug the trap and carbon dioxide can mask some of the early eluting compounds in the chromatogram.

After loading, the trap is often dry-purged with gas to remove excess water, methanol, and carbon dioxide that may have accumulated on the trap. This step is not always necessary, but many of the automated purge and trap concentrators allow for dry purging. Care must be taken not to blow any of the lighter VOCs off the trap during this step. During desorption the trap is rapidly heated and placed in series with the chromatographic column. The trap should not be heated above

TABLE 15.4 Common Adsorbents Used in Traps for VOCs

Adsorbent Packing	Properties
Tenax A	Hydrocarbons (b.p.>35°C), BTEX, nonpolar compounds, polar compounds poorly retained
Silicagel	Hydrocarbons (b.p.<35°C); retains polar compounds, but also adsorbs water and methanol
Activated-charcoal carbon molecular sieve	Light gases, absorbs carbon dioxide
OV-1	Light gases, no carbon dioxide; slightly polar column packing used in early traps; bleeds easily
Vocarb 3000	Broad range of VOCs, thermally stable to 250°C; hydrophobic
Vocarb 4000	Broad range of VOCs, thermally stable to 250°C; hydrophobic, but can be used for large molecules

the temperature limit of the trap packings. Tenax, for example, is a polymer of diphenyl oxide and begins to break down at temperatures above 250°C. Usually the purge and trap is plumbed so that the carrier gas from the gas chromatograph flows across the trap in the reverse direction from which it was loaded during the purge cycle. Figure 15.7 shows the gas flow diagrams during the purge and desorption cycles for a common purge and trap. Gas flows are switched using specially heated six-port valves.

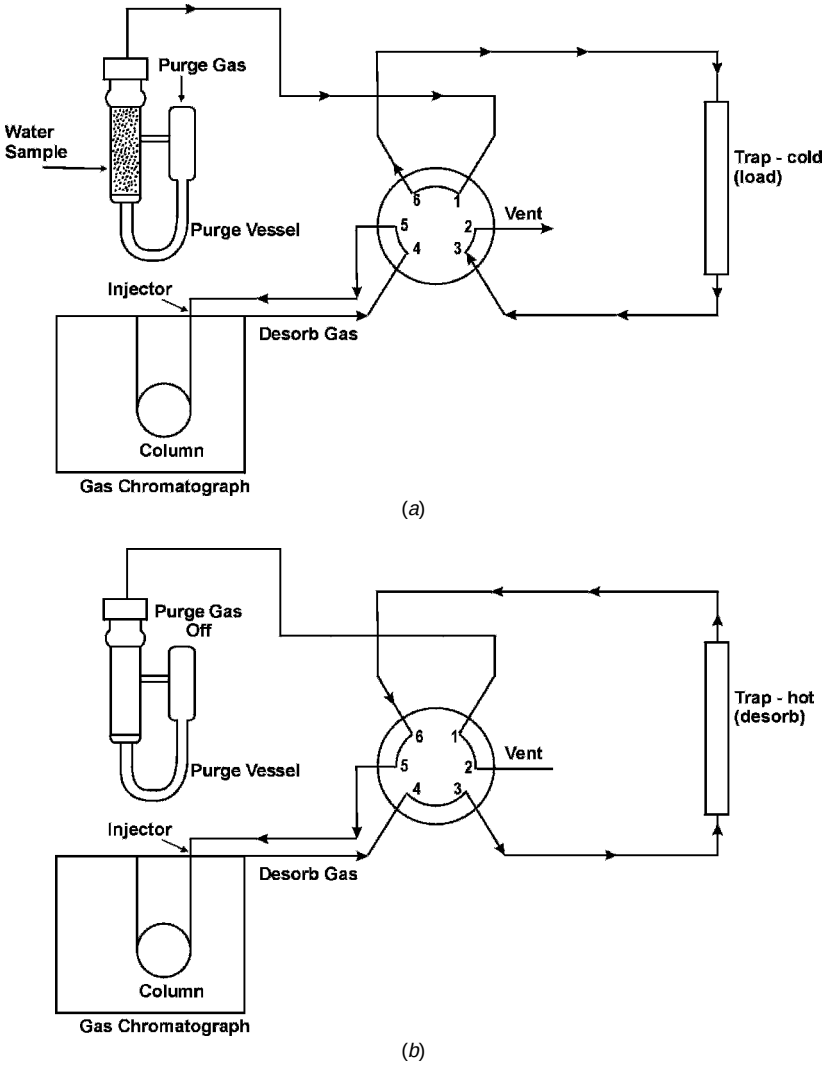


FIGURE 15.7 Schematic diagram of gas flows controlled by six-port valve during purging and trapping of VOCs from aqueous samples: (a) purging of water sample and loading of trap; (b) desorption of VOCs from trap onto gas chromatographic column.

Since the desorbing gas is also the carrier gas, the diameter of the chromatographic column limits the flow. Gas flows of at least 5–10 mL/min are needed to adequately desorb a standard trap in reasonable amount of time. These flows work well with a megabore capillary column (0.53 mm i.d.). However, if a narrow-bore capillary column (0.25 mm i.d.) is being used the flow during desorption is limited to 1–2 mL/min. A common way to interface the purge and trap with the gas chromatograph and overcome this problem is to split the flow coming from the trap at the injection port of the GC. Using the split/splitless injector found on most gas chromatographs, a split ratio of approximately 10–1 can be set to allow a flow of 10 mL/min across the trap during desorption and a flow of 1 mL/min through the column. Of course this occurs at the expense of diminished sensitivity, because a portion of the adsorbed analytes from the trap are vented.

15.6 EXTRACTION TECHNIQUES FOR SEMIVOLATILE ORGANIC COMPOUNDS IN AQUEOUS SAMPLES

The gas-phase extraction techniques discussed above are generally not applicable to the determination of semivolatile organic compounds in aqueous samples. Direct aqueous injection can be performed, but this technique, however, is not very sensitive. Often it is advantageous to transfer the semivolatile compounds into an organic phase. Once in the organic phase, these compounds can be concentrated and co-extracted organic material that could possibly interfere with the gas chromatographic analysis can be removed using a cleanup step. The classical liquid–liquid extraction (LLE) can be used to do this and will be discussed in the following section. Solid-phase extraction (SPE), which can also produce the same effect using the principles of reversed-phase liquid chromatography, will also be discussed. In SPE an aqueous sample is allowed to pass through a solid-phase adsorbent to trap the organic constituents that are present. These organic compounds are then eluted using a small volume of organic solvent.

15.6.1 Liquid–Liquid Extraction

In most environmental laboratories the classical liquid–liquid extraction using the separatory funnel is still the most used technique to extract semivolatile organic compounds, pesticides, and PCBs from aqueous samples. Methylene chloride (MeCl), although a potential health hazard, is one of the most commonly used solvents. Several physical properties make it advantageous to use. These properties are listed in Table 15.5.

Methylene chloride is significantly more dense than water, making it the bottom layer in the separatory funnel and easier to remove. The higher density contributes to a faster separation of the phases and makes the formation of emulsions less likely. It has a low boiling point; therefore, it is easy to evaporate when concentrating analytes or exchanging with another solvent. It is immiscible with water, and water is not very soluble in methylene chloride, making it easy

TABLE 15.5 Physical Properties of Methylene Chloride

Physical Property	
Density (20°C)	1.326 g/mL
Boiling point	39.75°C
Solubility in water	1.6% (w/w)
Solubility of water in MeCl	0.24% (w/w)
Polarity index	3.1
Flash point	none

Source: Adapted from References 38–40.

to dry when using a desiccant like anhydrous sodium sulfate. Because it has a moderate polarity most SVOCs, pesticides, and PCBs readily partition into it from the aqueous phase. Because it is chlorinated, it has no flashpoint and will not cause an explosion or fire.

The distribution that occurs for an organic analyte between two immiscible liquid phases can be described by the partitioning coefficient K_d, as shown here

$$K_d = \frac{C_o}{C_{aq}} \tag{15.5}$$

where K_d = distribution coefficient
C_o = concentration of analyte in organic phase
C_{aq} = concentration of analyte in aqueous phase

Analytes with higher distribution coefficients are more easily extracted. When an aqueous sample is placed into a separatory funnel and shaken with an aliquot of immiscible organic solvent, the fraction f remaining unextracted is

$$f = \frac{V_{aq}}{V_{aq} + K_d V_o} \tag{15.6}$$

After n successive extractions with a fresh aliquot of organic solvent, the analyte remaining in the aqueous phase is

$$f = \left[1 + K_d \left(\frac{V_o}{V_{aq}}\right)\right]^{-n} \tag{15.7}$$

taken from Reference 41.

USEPA SW846 Method 3510C for liquid–liquid extraction calls for the extraction of 1 L of water with three successive 60-mL portions of methylene chloride (13). If $K_d = 200$ for a particular analyte, we can use Equation 15.7 to calculate that after one extraction 92.3% of the analyte will be in the organic phase, after two extractions 99.4%, and after three extractions 99.9%. Three

serial extractions are usually normal for most liquid–liquid extractions using a separatory funnel.

The pH of the aqueous phase can be adjusted to form the nonionized species for an analyte or group of analytes. Phenols, for example, are best extracted at a low pH because the proton remains on the hydroxyl group. On the other hand, at elevated pH, amines are not protonated and partition more readily into the organic phase. Knowledge of the pK_a for an analyte is helpful in optimizing the pH for a liquid–liquid extraction. Most times in environmental analysis a large number of compounds are to be extracted and optimization for one analyte is not practical. When the liquid–liquid extraction is used for USEPA SW846 Method 8270, GCMS analysis of SVOCs, the water sample is pH-adjusted to a pH greater than 11 with sodium hydroxide and extracted with three successive 60-mL aliquots of methylene chloride. The pH is then taken to a pH less than 2 with hydrochloric acid and again extracted with successive 60-mL portions of methylene chloride. The base–neutral fraction and the acid fraction are often combined for the concentration step.

The continuous liquid-liquid extraction is often required on aqueous samples. It can be used if emulsions occur during the separatory funnel shakeout. Emulsions are more likely to occur when the density of the aqueous phase and the organic phase are nearly equal. Aqueous samples that are highly contaminated with hydrocarbons and are loaded with dissolved organic matter are leading candidates for emulsions. Sometimes emulsions can be broken by adding a salt to the aqueous phase and by mechanical manipulation. A high-density solvent such as methylene chloride is heated and allowed to condense in a reflux tube. The continuous extraction device is configured so that the methylene chloride condensate percolates down through the aqueous sample over an extended period of time (18 h). A very high ratio of the organic phase to the aqueous phase is achieved creating a highly efficient extraction. An example of a continuous liquid–liquid extraction apparatus is depicted in Figure 15.8.

The term *microextraction* is applied to a liquid–liquid extraction when the ratio of the organic phase to the aqueous phase (V_o/V_{aq}) is the range 0.05 to 0.2, as with the separatory funnel shake out and the continuous extraction described above. Sometimes, however, it is beneficial to use a smaller amount of solvent and perform a single extraction. Microextractions, when the ratio of the organic phase to the aqueous phase is in the range of 0.001–0.01, are usually simpler and faster to perform. Although not extracted quantitatively, the analyte is more concentrated in the organic phase than with the macroextraction. Let us go back to the example from above using the separatory funnel. We extracted 92.3% of an analyte with a distribution coefficient (K_d) of 200 from one liter of water using a single 60-mL portion of solvent. Assume initially that 10 mg/L of the analyte was present in the sample. The first extract (organic phase) would have the analyte present at approximately 167 μg/mL. Suppose instead that we extract 100 mL of the sample with 1.0 mL of sample. This can be done practically and easily by placing it in a 100-mL volumetric flask and adding 1.0 mL of solvent less dense than water (e.g., hexane) so that the solvent comes up in

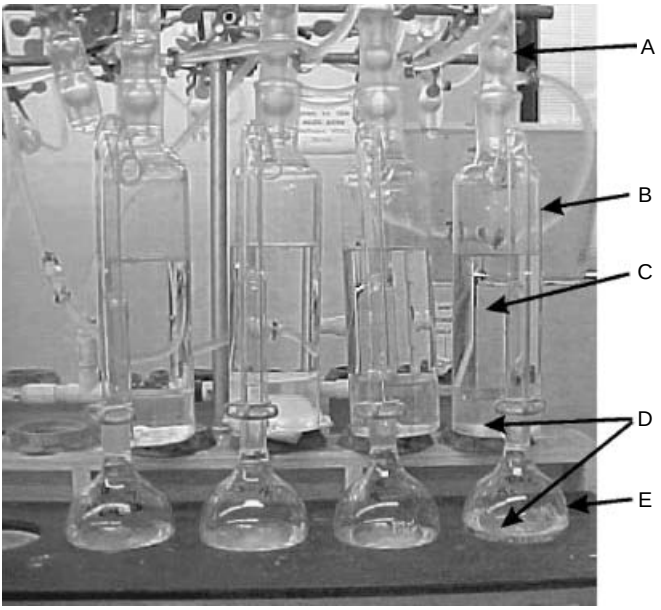


FIGURE 15.8 Digital photograph of continuous liquid–liquid extraction apparatus used for aqueous samples: A—reflux condenser; B—sample reservoir and extraction chamber; C—aqueous sample (1 L); D—methylene chloride, extraction solvent; E—500-mL round-bottomed flask in heating manifold.

the neck of the flask. After shaking the flask, we remove the solvent using a micropipette and transfer it into an autosampler vial. The concentration of the analyte in the extract is 670 μg/mL, approximately 4 times more concentrated than with the macroextraction. Although the amount of analyte removed from the aqueous sample is less, the analyte is more concentrated in the extract. The advantages of the microextraction are that much less solvent is required and the sample extract may not require evaporation to concentrate the analytes. USEPA Method 505 for the determination of pesticides in drinking water and Method 504 for the determination ethylene dibromide (EDB) in drinking water both use the microextraction technique (14).

15.6.2 Solid-Phase Extraction

Solid-phase extraction (SPE) is a form of liquid chromatography. During SPE a liquid sample is passed through a cartridge or disk containing several milligrams to several grams of a sorbent or solid phase. These sorbents are usually silica-based powders onto which chemical functional groups have been bonded and resemble those used for high-performance liquid chromatographic columns. The particles, however, are larger and range in size from 15 to 100 μm to allow rapid liquid flow under low pressures or to vacuum (e.g., in the range of 10 to 15 psig).

These cartridges have been popular in environmental analysis since 1978, when they became commercially available (42,43).

Solid-phase extraction (SPE) can be used to separate an organic analyte from the aqueous phase and to concentrate the analyte in a few milliliters of solvent. SPE also can be used to clean up a sample matrix and remove concomitant contaminants from the analyte. In some cleanups the analyte is absorbed onto the solid phase and the interferences pass through unretained. In the opposite cleanup strategy the solid phase retains the interferences and allows the analyte to pass through the cartridge with the mobile phase. In some environmental application, SPE performs all three roles—extraction, concentration, and cleanup.

The bonded phases used in the SPE cartridges range in polarity from the nonpolar phases like octadecyl silane (ODS or C18) to polar phases such as cyanopropyl or diol phases. Ion exchange phases are also available. Retention of the analyte onto an adsorbent is a function of the interactions between the adsorbent and the analyte and the sample solution. When an aqueous sample containing hydrophobic organic analytes is passed through a C18 sorbent the analytes are strongly retained because of nonpolar interactions. These nonpolar interactions are result of Van der Waals or dispersive forces (44). The more hydrophobic and less soluble the analyte the more efficient it will be extracted by these mechanisms (45). Reversed-phase chromatography occurs when the mobile phase is more polar than the stationary phase. Most environmental applications using SPE are reversed-phase separations.

Polar interactions can also occur between functional groups on the analyte and the polar functional groups on the solid sorbent (44). These include hydrogen bonding, dipole–dipole, induced dipol–dipole, and $\pi-\pi$ interactions. Functional groups, which demonstrate such interactions, are hydroxyls, amines, carbonyls, aromatic rings, double bonds, and groups with polar heteroatoms such as oxygen, nitrogen, sulfur, and phosphorus. Such interactions are common to all bonded silica because of the exposed silanol groups. Polar interactions are most significant in a nonpolar solvent. When a polar phase, such as silica, is used to extract polar compounds from a nonpolar solvent such as hexane, normal-phase chromatography is occurring.

Before a C18 cartridge or disk can be used to extract an aqueous sample, it must be properly conditioned and prepared for extraction. The sorbent is usually rinsed with a solvent such as methanol to wet the bonded phase and the silica backbone. It is then rinsed with several bed volumes of reagent water to remove the excess methanol. The sample is then loaded onto the cartridge. Usually the aqueous sample is pulled through the cartridge using a vacuum manifold as shown in Figure 15.9. Flowrates of 5–10 mL/min are common for SPE when cartridges are used. Faster flow rates of up to 100 mL/min are possible with disks. The sample size will depend on the analyte(s) and the aqueous sample. Large sample volumes or high concentrations of analytes can exceed the capacity of the solid-phase cartridge and cause the analytes to breakthrough and elute from the cartridge. Whenever a SPE method is developed, the breakthrough volume needs to be determined for the analytes of interest. Sediment or suspended matter

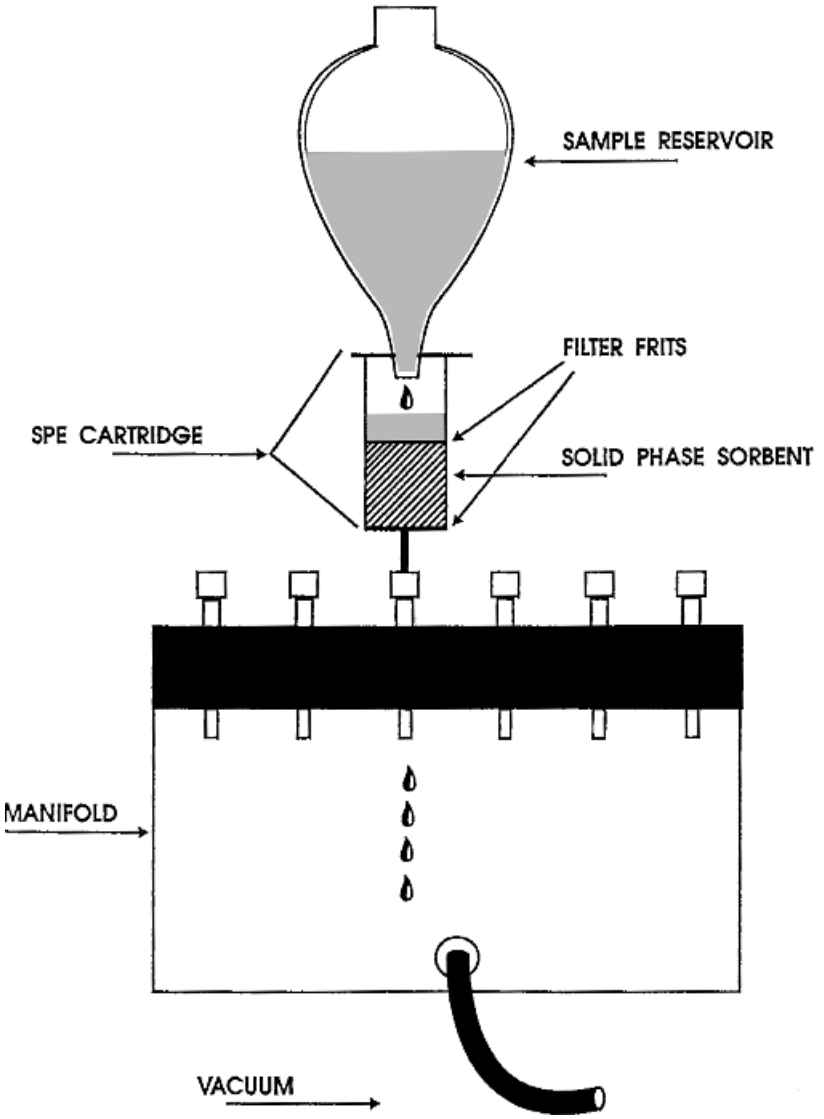


FIGURE 15.9 Diagram of a vacuum manifold used for solid-phase extraction (SPE) of liquid samples.

will also plug the cartridge and suspend the extraction process. Retention of the analyte is highly dependent on the sample matrix. It must be realized that other organic compounds present in the sample can compete for adsorptive sites on the solid phase and displace the analyte. Analytes, which are soluble in water, may be difficult to extract. As with the liquid–liquid extraction, the water sample may be salted to decrease the solubility of the organic analytes and increase their

retention on the solid phase. The sample matrix may also require pH adjustment to produce a neutral species if reversed-phase SPE is being practiced. After the sample has been loaded, it may be necessary to dry the cartridge or disk by pulling air through it, especially if a solvent immiscible with water is going to be used to elute the cartridge. Usually several milliliters of solvent are used to elute the cartridge or disk.

The Empore disks were developed as an alternative to the SPE cartridges (46). These disks resemble a membrane filter and are made of Teflon. They can be used with normal laboratory filtering glassware. The bonded silica particles are meshed in Teflon and offer a very large surface area. Because the disks have a larger cross-sectional top area than the cartridges, they are less likely to plug and offer the advantage of faster sample flowrates. USEPA Method 525.2 makes use of these disks for extracting semivolatile organic pollutants from drinking water for analysis by GCMS (47).

15.6.3 Solid-Phase Microextraction

Pawliszyn and the author first reported on the technique of solid-phase microextraction (SPME) in 1990 (48). Since that time SPME has been commercialized (Supelco, Bellefonte, PA) and the number of applications of SPME to environmental analysis has exploded (7,49,50). When used for gas chromatography the technique requires no solvent. Solid-phase microextraction can be used to extract analytes from an aqueous phase or the gas phase. The technique can be used to sample the headspace over water and soil samples.

Very little skill is required to practice SPME and the necessary equipment is minimal. A fused-silica fiber coated with a liquid–polymeric phase is located in the needle of a holder that resembles a syringe. The fibers are available in a variety of phases of varying polarity and film thickness (7–100 μm). The nonpolar polydimethylsiloxane (PDMS) is the most common. More polar phases such as Carbowax and acrylate are also used to coat the fiber. To perform SPME the needle of the holder is used to pierce the septum of the sample vial with the fiber withdrawn into the needle. The plunger of the needle is then depressed to push the fiber from the needle and expose it to the either the headspace or the liquid sample. Equilibration with sample can vary ranging from 2 to 30 min. Water samples are often stirred while the fiber is submersed. When sampling is complete, the fiber is withdrawn into the needle and it is removed from the sample vial. The needle is then inserted into the hot injection port of a gas chromatograph to desorb the analytes from the fiber for chromatographic separation. Desorption times and temperatures will depend on the liquid phase, the thickness of the coating, and the volatility and polarity of the analyte. Desorption times of two to three minutes and injection temperatures of 150–300°C are common. Higher-molecular-weight compounds will demonstrate peak tailing if the fiber is too thick. The fiber is reusable. Over 50 injections per fiber are not uncommon. An autosampler is commercially available to perform unattended SPME. Obtaining accurate quantification with SPME can be very difficult. Matrix composition, pH, temperature, and extraction times must be strictly controlled (51).

15.7 EXTRACTION TECHNIQUES FOR SEMIVOLATILE ORGANIC COMPOUNDS IN SOIL AND SOLID SAMPLES

Soils and sediments represent the majority of solid environmental samples. Soil is made up of a number of components. A typical arable soil contains about 5–6% organic matter and 95% inorganic matter. Some soils, such as peat, can contain as much as 95% organic matter, while others hold less than 1%. Humic substances represent about 80% of the organic matter in soil and contain residue from plant decay, consisting of mainly carbon, hydrogen, and oxygen. Three major humic fractions exist in soil: humic acid, fulvic acid, and humin. Humin is not water-soluble under any condition and fulvic acid is water-soluble over the entire pH range. Humic acid is water-soluble only at a pH $\leq$ 1 (52). Fats, resins, and waxes constitute only several percent of the soil's organic matter. Polysaccharides, such as cellulose, starches, hemicellulose, and gums, provide nutrients for microorganisms that live in the soil. Organic compounds that contain nitrogen and phosphorus are also found in soil and include amino acids, amino sugars, phosphate esters, inositol phosphates, and phospholipids. The inorganic component of the soil may consist of silicates, quartz, metal oxides, and calcium carbonate (53). Besides soil and sediment, other solid, environmental samples include flyash from the incineration of municipal and hazardous waste, industrial and wastewater treatment sludges, and tissue samples from plants and animals.

The most common means of isolating semivolatile and nonvolatile organic compounds from soil and other solid samples is the liquid–solid extraction. Since soils are not as homogenous as waters, care must be taken to ensure that the sample is homogenous. Often large clumps of soil are crushed, large pieces of organic debris are cut into smaller pieces, and the soil is "coned and quartered" to ensure uniformity. This blending and manipulation of the soil is possible when volatile compounds are not of concern. The solvent must be able to penetrate the soil for the extraction process to be efficient. Water can prevent a hydrophobic solvent from penetrating the soil. A drying agent, such as sodium sulfate, is often added to the soil and a water-soluble solvent (e.g., acetone) is added to the extracting solvent. For the solvent to be successful in isolating a given analyte from the soil matrix, the analyte must first be sufficiently soluble in that solvent and secondly, the solvent must be able to overcome the adsorptive forces which bind the analyte to the soil. Normally the surface area of soils range from 25 to 100 m^2/g (19).

The forces that can bind the organic contaminants to the soil surface include hydrogen bonding, charge transfer, ligand exchange, ion exchange, interactions of direct and induced dipoles, and chemisorption. Soil organic matter dominates the adsorption of neutral organic compounds. Evidence also exists that when soils containing organic pollutants are subject to wetting and drying cycles, as occurs with natural weathering, compounds become more difficult to desorb from the soil surface. The analyte must sufficiently partition into the organic solvent phase from the soil: (1) the organic analyte must be desorbed from the solid particle and (2) if the analyte is located within a pore in the soil, it must diffuse

through the solvent located inside a particle pore and then transfer to the bulk of flowing fluid (54).

The classical chromatographic distribution constant K_D can be used to express the equilibrium condition, which occurs when the analyte partitions between the extracting solvent and the soil

$$K_D = \frac{C_s}{C_o} \tag{15.8}$$

where C_s is the concentration of the analyte in the soil and C_o is the concentration of the analyte in the organic solvent.

Any steps to decrease K_D will serve to increase the extraction efficiency of the method. Two of the most common methods of extracting pesticides from soils are the sonication and Soxhlet extraction. USEPA endorses both methods for the extraction of soils.

15.7.1 Soxhlet Extraction

USEPA SW846 Method 3540C outlines the Soxhlet extraction. (13). The soil (10−30 g) or solid sample is mixed with a drying agent such as sodium sulfate and placed in a cellulose extraction thimble between two plugs of glass wool. The pH of the soil is not normally adjusted, but surrogate standards are added to monitor the efficiency of the extraction process. The thimble containing the soil is then placed in a Soxhlet apparatus. The extraction solvent (300 mL) is added to a round-bottomed flask that is connected to the Soxhlet apparatus. An equal volume mixture of methylene chloride and acetone is used for SVOCs, pesticides, and PCBs. When heated, the solvent evaporates and rises into the cooled condenser where it condenses and drips down, percolating through the soil, which is contained in a porous cellulose thimble. When the solvent in the upper chamber reaches a level that is above the side arm, the solvent siphons into the lower flask. This process continues in a cyclic fashion. The temperature is usually adjusted so that at least 6 cycles occur per hour. The extraction is usually conducted over an extended period of 16−24 h. During the cycling process, the heavier and less volatile compounds accumulate in the round-bottomed flask and essentially fresh solvent condenses and percolates through the soil, producing a very thorough extraction. The glassware and equipment required for the Soxhlet extraction is relatively inexpensive, but the technique does require large volumes of solvent. Significant manual effort is required to set up a batch of extractions, but they can be left unattended once they begin. Because the extraction solvent is hot, thermally labile analytes can break down. A Soxhlet apparatus used to extract solid samples is pictured in Figure 15.10.

USEPA Method 3541 is an automated version of the Soxhlet extraction (13). The soil sample is prepared in the same way as with the Soxhlet extraction, and similar solvents are used. The automated Soxhlet extractor (Soxtec) allows the extraction thimble to be lowered into the boiling solvent for one hour. The automatic Soxhlet extractor achieves equivalent extraction efficiency with the

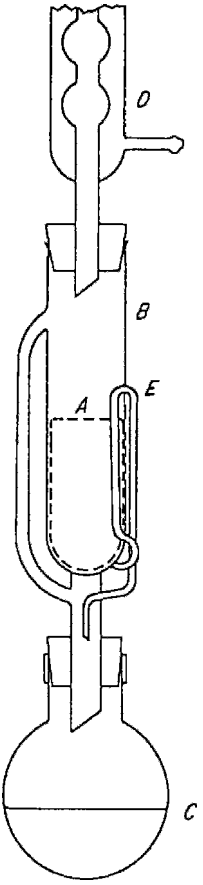


FIGURE 15.10 Soxhlet extraction apparatus used for extracting solid samples: A—sample thimble; B—extractor body; C—extraction solvent; D—reflux condenser; E—siphon return.

manual extraction in only 2 h. It also can automatically evaporate the solvent and concentrate the analytes. The disadvantage is that it is an expensive device.

15.7.2 Ultrasonic Extraction

During the sonication extraction, ultrasonic vibrational energy is used to enhance the liquid–solid extraction. The generator or power supply converts 50–60 Hz alternating current to 20 kHz high-frequency electrical energy. A converter, whose main component is a lead zirconate titanate piezoelectric crystal, translates this electrical energy into high-frequency mechanical energy (55). The converter vibrates in a longitudinal direction and transmits the motion to the horn tip immersed in the extraction solution. The sonic or ultrasonic wave traveling through the solution consists of alternate compressions and rarefactions. If the

amplitude of the wave is high enough, cavitation results. Cavitation is the rapid and repeated formation of microbubbles (150 μm diameter at 20 kHz) in the solution, which implode and propagate the shock. These bubbles take many cycles to grow to what is known as a *resonant size*, at which point they collapse, instantaneously producing high local pressures of 20,000 atm (56). The mechanical shock is transmitted only a few micrometers, however.

Most of the early applications of sonication were biological and concerned with the use of ultrasound to rupture and shear cells and with the bactericidal effect of ultrasonics (57). This ultrasonic energy, however, was found beneficial for the liquid–solid extraction of soils. The major benefit of applying ultrasonics to soil extractions is the disaggregation of soil particles, which occurs during the extraction procedure as the result of the sonic energy. This increases the surface area of the soil and allows the extractant to further penetrate the soil matrix. Also, the mechanical agitation, which occurs during the sonication extraction, rapidly exchanges the solvating layer surrounding the soil particles and aids in the mass transfer of the analyte from the solid surface into the solution. In USEPA SW846 Method 3550B for the sonication extraction, the soil is mixed with anhydrous sodium sulfate and extracted serially with three portions of 1–1 acetone and methylene chloride. During the extraction the soil is pulsed at 50% duty cycle so that the energy to the horn is on 50% of the time. The organic solvent is decanted from the soil, filtered, and collected after each extraction (13).

Following either the sonication or the Soxhlet method, the extract is passed through a sodium sulfate column to remove any water in the extract. The organic solvent must then be evaporated to enrich the analyte concentration in the extract if ppb detection levels are to be achieved. The sonication extraction is very labor-intensive and requires large amounts of solvent. Because of the decanting and filtering steps, the analyst can be exposed to solvent vapors if proper precautions are not taken.

15.7.3 Pressurized Fluid Extraction

The term *pressurized fluid extraction* (PFE) was adopted in the USEPA SW846 Method 3545 to avoid showing partiality to a commercial company. However, the term most used by the environmental community for this technique is *accelerated solvent extraction* (ASE), which was penned by those who first described it and manufactured the first instrumentation to perform this technique (58,59). Soil samples are prepared for extraction by air-drying or by mixing with diatomaceous earth. Sodium sulfate can be used, but is a poor choice because it has been known to clog cell frits and the extractor lines. After desiccation, the sample is often pulverized or ground so that it is a free-flowing powder and loaded into the extraction cell. Cells are stainless-steel tubes with screw-on end caps and available in sizes of 11, 22, and 33 mL (60). The end caps have stainless-steel frits that prevent particles from escaping from the cell and peak seals that form a pressure-tight cell when the cell is placed online for extraction. Once the sample has been placed in the extraction cell, it is loaded onto a carousel that is capable

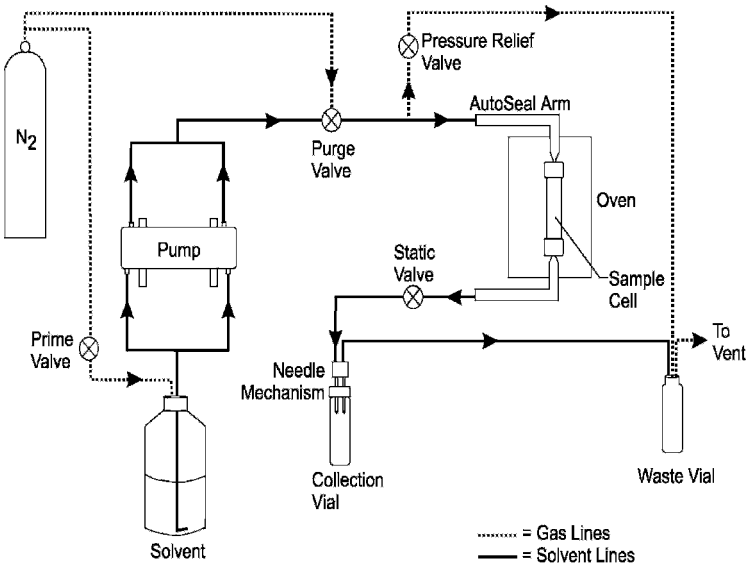


FIGURE 15.11 Schematic diagram of the accelerated solvent extractor (courtesy of Dionex Corp.)

of holding 24 cells. A schematic diagram of the accelerated solvent extractor is shown in Figure 15.11.

Any of the common laboratory solvents can be used in the extractor, but the final choice will depend on the analytes that are to be extracted. Fritzpatrick and Dean described a method for selecting the optimum solvent for the extraction of DDT (and metabolites) and pentachlorophenol (PCP) from soil when using ASE; they broke the Hildebrand solubility parameter down into three components, to optimally select methylene chloride for DDT and a mixture of methylene chloride and acetonitrile for PCP (61).

An equal volume mixture of methylene chloride and acetone is a good choice for most SVOCs since the mixture is used in most other environmental extractions of soil. The instrument used for ASE can control a number of extraction conditions. The conditions that are recommended by USEPA Method 3545 are given below:

Oven temperature	100°C
Pressure	1500–2000 psi
Static time	5 min
Flush volume	60% of the cell volume
Nitrogen purge	60 seconds at 150 psi
Static cycles	1 cycle

During the extraction cycle, the cell is removed from the sample carousel and sealed at both ends by the autoseal arms in the oven. The pump fills the cell with

solvent, bringing the pressure to the setpoint while the oven brings the cell to the extraction temperature. After thermal equilibrium has been established, the sample is held static for a period of time so that the hot solvent can soak the sample. The pump maintains the pressure during this time, but no solvent exits the cell. After 5 min the solvent is vented from the cell and collected in a glass vial. A second carousel holds all of the collection vials, usually one per sample cell although multiple fractions can be collected. The sample cell and the lines to the collection vial are then rinsed with an additional volume of solvent. After the cell has been flushed, nitrogen gas is used to purge any remaining solvent from the extraction cell and the lines into the collection vial. The accelerated solvent extractor can be programmed to repeat this cycle several times on a sample. For an extraction of 10 g of soil, a collection volume of 15–20 mL of solvent would be typical.

Increasing the temperature of the solvent improves the efficiency of the extraction for a number of reasons. The solubility of most analytes increases at higher temperatures. The rate of diffusion of the analytes in the solvent also increases. The viscosity and surface tension of the extraction solvent also decreases, which allows better penetration into pores and interstitial spaces of the soil. The solubility of water in nonpolar solvents increases at higher temperatures and allows better penetration of the solvent into cell pores. Weakening and disruption of van der Waals forces between the matrix and the analytes occurs at higher thermal energy. Pressure seems to have no significant influence on the recovery of analytes, although increased pressure does serve to keep the solvents from boiling and from a practical standpoint, forces the solvent into the soil pores (62,63).

The advantages of ASE are an efficient extraction that compares favorably with the Soxhlet and sonication extractions, the use of considerably less solvent than in the classical techniques, and automated operation once the sample cells are loaded. Some of the disadvantages of ASE are the high cost of equipment, the extensive labor of preparing the sample and loading it into extraction cell, and the possibility of instrument failures such as plugging of solvent lines, leaks, and mechanical jams.

15.7.4 Supercritical Fluid Extraction

In the early 1990s it appeared that supercritical-fluid extraction was going to be the future method of choice for extracting environmental soils and solid samples. SFE showed promising recoveries for many environment analytes and used very little solvent (64). As of 2001, it had not gained the widespread use that was predicted (7). SFE is very similar to the ASE technique described above, except that a supercritical fluid is used for the extraction rather than a solvent. Any pure substance that is above its critical temperature (T_c) and critical pressure (P_c) is defined as a supercritical fluid. The most frequently used extraction fluid is CO_2. If CO_2 is compressed to a pressure above 72.9 atm and heated to above 31.3°C, it becomes a supercritical fluid and exhibits physical properties between those of a gas and a liquid. Carbon dioxide is used most frequently in SFE as an extraction

fluid because it has a number of favorable physical properties (65). It is unreactive and will prevent oxidation from the air during SFE. Moreover, carbon dioxide has low toxicity and poses virtually no safety threat in the laboratory other than displacement of oxygen in the air. It also has a relatively low critical temperature and pressure, making it easy to obtain supercritical conditions and useful for the extraction of thermally labile analytes. Finally, CO_2 is readily available at high purity for minimal cost. A list of supercritical fluids and their physical properties are listed below in Table 15.6.

Some of the fluids listed in Table 15.6, however, have properties that make them undesirable for SFE. For example, ethane is a highly flammable gas, making it dangerous to use in the laboratory at high pressures. Nitrous oxide is a strong oxidizer and has been known to form explosive mixtures with certain organic samples and modifiers. Some of the more polar fluids, such as methanol and ammonia, have high critical points, making them impractical for SFE. Supercritical ammonia is also a very aggressive solvent, which is difficult to pump and would attack conventional SFE instrumentation. Some of the fluids (e.g., fluoroform-HCF_3) are good solvents for classes of compounds, but their high costs limit their use.

Some physical properties of supercritical fluids are in between those of typical gases than liquids. For example, the viscosity of supercritical fluids is about an order of magnitude lower (10^{-4} vs. 10^{-3} N·s/m^2) and the solute diffusivity of supercritical fluids is an order of magnitude higher (10^{-4} vs. 10^{-5} cm^2) than for liquid solvents (64). These properties of viscosity and solute diffusivity contribute to improved mass transfer for solutes in the supercritical state, and, therefore, speed extraction rates. The density of carbon dioxide can be increased to densities

TABLE 15.6 Physical Parameters of Selected Supercritical Fluids

Fluid	T_C (°C)	P_C (atm)	ρ_C (g/mL)	V_C (mL/mol)	Dipole Moment (debyes)
CO_2	31.1	72.8	0.468	94	0.00
N_2O	36.4	71.5	0.452	97	0.17
H_2O	374.1	217.6	0.322	56	1.85
Methanol	239.4	79.9	0.272	118	1.70
NH_3	132.3	111.3	0.235	72	1.47
Ethane	32.4	48.3	0.203	140	0.00
Ethene	10.0	51.2	0.227	124	0.00
Benzene	288.9	48.3	0.302	259	0.00
CHF_3[a]	25.9	47.7	0.516	136	1.62
CCl_2F_2[b]	11.7	39.4	0.557	217	0.17
$CHClF_2$[c]	96.0	49.1	0.524	165	1.42

[a] Freon 23.
[b] Freon 12.
[c] Freon 22.

Source: Adapted from References 64 and 65.

higher than those of some liquids by increasing the pressure. For example, at a pressure of 400 atm and temperature of 50°C, CO_2 has a density of 0.924 g/mL. Hexane and methanol have densities at 25°C of 0.660 g/mL and 0.791 g/mL, respectively (65). The solvent strength of liquids does not change very much with extraction conditions. However, in SFE over a practical pressure range, increasing pressure and, therefore, increasing the density of the fluid can increase the solvent strength of a supercritical fluid. The solubility of a solute in a supercritical fluid, therefore, is increased with this increased density.

Temperature also affects solubility. Generally, varying temperature at a constant pressure will reduce density and, therefore, the solvating power of a supercritical fluid. Increasing temperature, therefore, could reduce extraction efficiency. Generally speaking, supercritical fluids are more effective extracting agents when the temperature is above the melting point of the solute and it is a liquid phase (65). This increases solubility because the intermolecular forces are less in the liquid state than in the solid state and are easier to overcome. Also, mass transfer of the solute into the supercritical fluid is increased because of increased diffusion rates at higher temperatures.

One limitation of carbon dioxide as an extractant is its polarity. In its supercritical state and at low densities, CO_2 has a polarity close to that of hexane. Even at extremely high pressures the solubility parameter may not approach that which is required to solubilize and extract polar analytes. This limitation can be overcome by the use of another extraction fluid, which is more polar, or by adding a polar modifier to the CO_2. The most commonly used modifier with CO_2 has been methanol. Increased solubilities and recoveries of polar analytes have been reported when a polar modifier is added to a less polar supercritical fluid (66–68). The ability of the supercritical fluid to dissolve a particular analyte is not the only factor, which affects extraction efficiency. The degree to which the analyte partitions into the supercritical fluid from the solid-sample matrix depends greatly on the sorptive and active sites on the solid matrix and the polarity of the solute (64,69). The addition of a polar modifier or entrainer, such as methanol, to a supercritical fluid such as CO_2, not only increases the solubility of polar analytes in the supercritical fluid, but also may help block sorptive sites on the surface of the sample matrix.

Commercial instrumentation has been available for approximately 15 years to perform analytical-scale SFE. A schematic diagram of a basic SFE system is shown in Figure 15.12. A high pressure pump, usually a stainless-steel syringe pump or reciprocating pumps used in HPLC, is the heart of the system and is required to achieve the high pressures (up to 450 atm) and maintain flowrates necessary to perform SFE. The soil or solid sample is placed in a stainless-steel extraction vessel, which is usually cylindrical and may vary in size from several microliters up to 50 mL or more. A desiccant, such as sodium sulfate or hydromatrix, is often mixed with the sample before it is placed in the extraction vessel. Clean sand is often used to fill any void volume that may be present in the extraction cell. An oven or a heater block controls the temperature of the extraction. Stainless-steel tubing ($\frac{1}{16}$ in.) usually connects the pump and the extraction

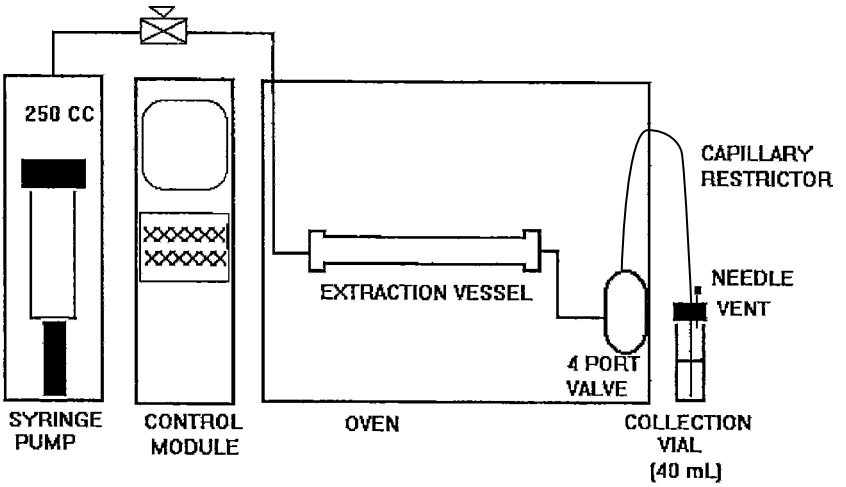


FIGURE 15.12 Schematic diagram of commercial supercritical-fluid extractor.

vessel. A restriction device, often a length of fine-bore capillary fused-silica tubing (10–100 μm i.d.), is used to vent the supercritical fluid to atmosphere. Such devices are referred to as *linear restrictors*. Other devices such as micrometering valves or specially electronically controlled valves with diaphragm apertures to regulate flow are also used instead of the capillary restrictors. The restrictor serves to help control the backpressure across the extraction cell and maintain supercritical conditions. If the restrictor diameter is too wide, the pump might not be able to maintain the desired extraction pressure and the CO_2 may be vented too rapidly to efficiently collect the extracted analyte. By application of the ideal-gas law, a supercritical fluid at 400 atm pressure when vented to 1 atm pressure will experience a 400-fold increase in volume. Therefore, a pumping rate of 1 mL/min over the sample at 400 atm translates to a gas flowrate of 400 mL/min. Extractions can be performed in the static or dynamic mode. In the static mode the supercritical fluid does not exit the extraction vessel and soaks the sample. In the dynamic mode the supercritical fluid flows over the sample and vents through the restrictor. An extraction may incorporate both the static and dynamic mode before completion. Besides the kinetics of mass transfer in SFE, the extraction time will be dependent on the sample size, the pumping capacity of the instrument, and the restrictor diameter. Generally SFE requires less than an hour per sample to complete. Often commercial SFE instruments are automated and controlled by a computer or microprocessor.

Several reasons exist for why SFE has not reached widespread use. Larger sample sizes are not practical on analytical-scale instrumentation. Limitations on flow of the supercritical fluid over the sample create longer extraction times. Sample sizes of 1–2 g place analytical-scale SFE at its upper limit. Many times the detection limits required by USEPA methods can not be achieved by smaller sample sizes (e.g., 1–2 g). Carbon dioxide is nonpolar and is really the only practical

supercritical fluid. It is best suited for extracting nonpolar analytes although modifiers can be added to extend the polarity range of analytes that can be extracted. SFE is very suited for some specific applications, such as extracting PCBs from a fatty matrix like fish, but does not do well in extracting large numbers of priority pollutants of differing polarity from a variety of soils. Maintaining SFE instrumentation and using it on large numbers of samples is challenging and requires a good deal of expertise and mechanical aptitude. High pressures and gases make leaks inevitable. Soils and extracted matter can plug lines and the restriction device. Two SFE methods are contained in USEPA SW846. Method 3560 is limited to extracting petroleum hydrocarbons from solid materials, and Method 3561 is for extracting PAHs from solid materials (13).

15.7.5 Miscellaneous Extraction Methods

Microwave-assisted extraction (MAE) is new technique that is gaining recognition and can be used to extract analytes from solid samples (70,71). Solid samples are placed in closed Teflon vessels with solvent. Microwave energy is used to heat the solvent, which accelerates the extraction and dissolution kinetics. Since the extraction vessel is closed, increased pressure prevents the solvent from boiling. Much of the discussion of the ASE theory in Section 15.7.3 would apply to MAE. The major difference is, of course, that the heating process is accomplished using microwave energy. No evidence seems to exist that the microwave energy adds any efficiency to the extraction other than heating the solvent, which increases the speed and efficiency of the extraction.

Thermospray nebulizers can be used to extract SVOCs from aqueous samples. When several thermospray probes simultaneously deliver solvent and sample into a cooled extraction vessel an efficient extraction can occur because of the increased exposure of the phases. Farrel and Pacey built a device called a thermospray liquid–liquid extractor (TSLLE). Using the TSLLE and methylene chloride they evaluated aqueous mixtures of SVOCs and obtained recoveries ranging from 80 to 100% during a single, 1-h cycle. The aqueous sample was delivered at 4 or 5 mL/min, and the methylene chloride was delivered at 2 or 3 mL/min. through heated capillaries into the chilled extraction vessel. The system was vented above a chilled condenser, and a stopcock at the bottom of the vessel allowed for phase separation of the methylene chloride after extraction (72).

The vacuum distillation technique can be used to extract VOCs that have a boiling point less than 180°C. USEPA SW846 Method 5032 describes the technique that can be used for a variety of liquid and solid matrices including animal tissue (13). The sample is place in a flask and distilled at a reduced pressure (10 Torr, vapor pressure of water) and ambient temperature. The distillate passes over a chilled condenser to remove water and compounds with high boiling points. The remaining distillate passes through the chilled condenser and is trapped in a cryogenically cooled collection tube. After collection, the tube is thermally desorbed directly into a gas chromatograph. Heavier contaminates such as oily residues and fats remain behind in the sample reservoir and do not

pass through the condensing coil. Between samples the condensing coil is heated to remove contaminants.

Azeotropic distillation is used as an extraction technique for volatile and polar analytes that are soluble in water. An azeotrope is mixture of two substances that behave like a single substance. A number of volatile ketones, aldehydes, alcohols, amines, and acetates that form azeotropes with water can be extracted from aqueous samples using this technique. USEPA SW846 Method 5031 describes both a macrodistillation and a microdistillation (13). In the macrodistillation one liter of water is buffer to pH 7 and the appropriate surrogate standards are added. The sample is brought to a boil and the distillate is collected after it passes through a cooled condenser in a special distillate chamber offset at the bottom of the condenser. The distillation is allowed to occur for about an hour. The azeotropic VOCs are condensed from the rising steam and concentrated into the aqueous distillate. In the microdistillation approximately 40 mL of sample is distilled through a fractionation column containing glass beads. Approximately 100–300 μL of aqueous distillate are collected. The VOCs are determined by GCFID or GCMS using direct aqueous injection.

15.8 CONCENTRATION STEP FOR SEMIVOLATILE ORGANIC COMPOUNDS

To achieve trace level analysis (ppb and ppm range) and to obtain the detection limits often required by regulatory agencies, the analytes present in an extract must be concentrated into a smaller volume. For the USEPA SW846 Method 8270, the GCMS method for SVOCs in water a concentration factor of 1000 is realized between the extraction and concentration step (13). After the extraction step a volume of approximately 350–400 mL of solvent will need to be concentrated to a volume of 1.0 mL to obtain this factor. Most environmental methods for SVOCs using gas chromatography use capillary columns that are either 250 or 320 μm in diameter. Normally this limits the injection size to one or two microliters unless special injection techniques, such as the large-volume injection (LVI) are practiced. The most common way to concentrate semivolatile analytes in an organic solvent is by evaporating the solvent. Unfortunately, this will also concentrate any coextracted interferences.

15.8.1 Evaporative Techniques

The most common way to evaporate solvent extracts that are more than 50 mL in volume is to use the Kuderna–Danish (K-D) apparatus. Before evaporation, water and soil extracts are usually dried by passing them through a column of anhydrous sodium sulfate, which has been baked to remove any organic impurities. Water can be especially troublesome when small volumes are reached during the solvent evaporation. During evaporation the solvent sample is placed into the K-D apparatus along with several boiling chips. The K-D apparatus has a graduated ampule, which is attached by a tapered, ground-glass joint to the bottom

of a 500-mL evaporator flask, to accurately measure the concentrated solution. A Snyder column, which contains three freely fitting glass spheres, is attached to the top of the evaporator flask. The K-D apparatus is placed on a hot water bath (80–90°C) so that the ampule is partially immersed in the hot water and the rounded surface of the evaporative flask is heated by the steam. The vertical position of the K-D apparatus is adjusted vertically and the evaporation should take 10–15 min to complete. During the distillation, the glass balls in the Snyder column should chatter, but the chambers between the balls should not flood with solvent. When the apparent volume of solvent remaining in the ampule appears to be approximately 1–2 mL, the K-D apparatus is removed from the steam bath and allowed to cool. After cooling the sample volume will increase because of the condensation of solvent vapors. Normally an increase of 5–10 mL is observed. If a solvent exchange is required, the Snyder column is removed and approximately 50 mL of the exchange solvent is added. The evaporation is repeated until the apparent volume reaches 1–2 mL. Adjustments may be required on the temperature and level of the water in the steam bath if the exchange solvent has a significantly different boiling point.

Because solvent evaporation is often required, the ampule containing usually 5–10 mL of solvent is removed from the bottom of the K-D, and a smaller two-ball Snyder column is attached. After the priming the micro-Snyder column a small of amount of solvent, the evaporation process described above is continued until the desired analytical volume is achieved. Normally the volume is taken to slightly below the desired volume and then adjusted to the exact volume with solvent. During the evaporation process it is important not to let the ampule go dry, or more volatile analytes will be lost. Usually surrogate standards have been added during the extraction step that can serve to monitor the evaporation step. For example, 2-fluorophenol is added to samples that require USEPA Method 8270, the analysis of SVOCs by GCMS. This compound elutes early on the total-ion chromatogram and has volatility comparable to the lighter SVOCs listed in the method. Low recoveries of this surrogate standard can indicate a problem with the evaporation process. Sometimes a nitrogen blowdown is used instead of a micro-Snyder column for the second step of the evaporation process. The concentrator ampule containing 5–10 mL of solvent is placed in a warm-water bath (approximately 35–40°C). The solvent is then evaporated to the desired volume under a gentle stream of clean nitrogen gas. A commercial apparatus that is commonly used to perform this task is called an *N-EVAP*.

Commercial equipment can be purchased that can perform the entire evaporation process in a single step. These devices require a gas supply, usually nitrogen, and have a self-contained thermostatted temperature bath. The solvent is placed in a cylindrical glass vessel that has a tapered bottom with a tubelike protrusion coming from the bottom. The solvent warmed by the temperature bath and evaporated by stream of nitrogen (40–50 mL/min) that vortexes over the surface of the liquid. When the liquid reaches the bottom of the vessel and drops into tube, the gas stream is switched off and the device beeps to alert the analyst that the process is complete. These devices require large quantities of nitrogen gas.

15.8.2 Solid-Phase Enrichment

When reversed-phase liquid chromatography is the mechanism at work during solid-phase extraction, analyte enrichment occurs. If water, a polar solvent, is passed through a nonpolar solid phase such as C18, hydrophobic nonpolar analytes will adsorb onto the C18. A small quantity of organic solvent can be used to flush the solid phase. For example, in Method 525.1 for determining SVOCs in drinking water, a one-liter water sample is passed through a C18 solid-phase cartridge or disk. Methylene chloride and ethyl acetate are used to elute the disk and rinse the sodium sulfate drying column. Approximately 20–25 mL of solvent are used. The analyte concentration is enriched by a factor of 40–50 times through the transfer to an organic phase. The volume of the solvent is further reduced to a volume of 1 mL by blowing it down under a gentle stream of nitrogen.

Normal-phase chromatography can also be applied to concentrate polar analytes that are dissolved in a nonpolar solvent, such as hexane. The hexane can be passed through a polar solid phase such as silica, Florisil, alumina, or a diol to adsorb the polar analyte. The polar analyte can be eluted with a smaller volume of a more polar solvent (e.g., ethyl acetate). Normal-phase SPE is not used very much for trace enrichment in environmental analysis, but is useful for matrix isolation and cleanup techniques.

15.9 CLEANUP OF SAMPLE EXTRACTS

Soil and water extracts can contain a variety of coextracted organic compounds that can interfere with the practice of good chromatography. Interferences can cause extraneous peaks on the chromatogram that can hide target analytes or hamper accurate integration. Large "hump-o-grams" can appear on the chromatogram, because of coextracted hydrocarbons and waxes. High-molecular-weight, nonvolatile compounds, even though they will not pass through the gas chromatographic column and show a response at the detector will deposit as a residue in the injector and the column. Peak tailing, a loss in resolution, and a change in the response factors of analytes can force maintenance and recalibration. Usually soil extracts require more cleanup than do aqueous samples. In some cases, a sample will require several cleanup steps in order to produce an acceptable chromatogram. The following sections discuss some of the common cleanup techniques that can be used on environmental samples.

15.9.1 Gel Permeation Chromatography

Gel permeation chromatography (GPC) is a universal technique for a broad range of SVOCs and pesticides. GPC is a form of size exclusion chromatography. Large molecules with higher molecular weights are not retained in the pores of the stationary phase because they are too big. These compounds spend more time in the mobile phase and elute first. Molecules that are smaller fit into the pores and reside longer in the stationary phase and elute from the column last.

The separation is based on molecular size. When GPC is practiced as a cleanup technique, marker compounds are injected to determine the fraction of the GPC chromatogram that will be collected to include the analytes of interest. USEPA Method 3640A describes the GPC cleanup for sample extracts. In the method a glass column (700 × 25 mm) is packed with divinylbenzene-styrene copolymer beads that are swelled in methylene chloride. Methylene chloride is the mobile phase and is pumped at 5 mL/minute. A calibration solution containing corn oil, bis(2-ethylhexyl) phthalate, methoxychlor, perylene, and sulfur is injected to determine the fraction that is to collected. A UV detector set at 254 nm is used to produce the chromatogram shown in Figure 15.13. Collection of the eluent from the column begins after the elution of the corn oil and before the elution of the bis(2-ethylhexyl) phthalate. Collection continues until after the perylene elutes and is stopped before the elution time of the sulfur peak. Prior to injection the sample extract is concentrated by evaporation to 10 mL. Then 5 mL is injected into the gel permeation column. Collection volumes of 50–60 mL are common and a twofold dilution of the sample is realized by the technique. GPC removes heavier fats, oils, large nonvolatile organic compounds, and elemental sulfur from the sample extract. System performance rather than the appearance of chromatograms often measure the effects of the GPC cleanup.

15.9.2 Acid–Base Partition

The concept of acid–base partitioning has been introduced above in Section 15.6.1. This concept can be used as a cleanup technique to separate acidic or basic compounds from each other or from neutral organic compounds. In this

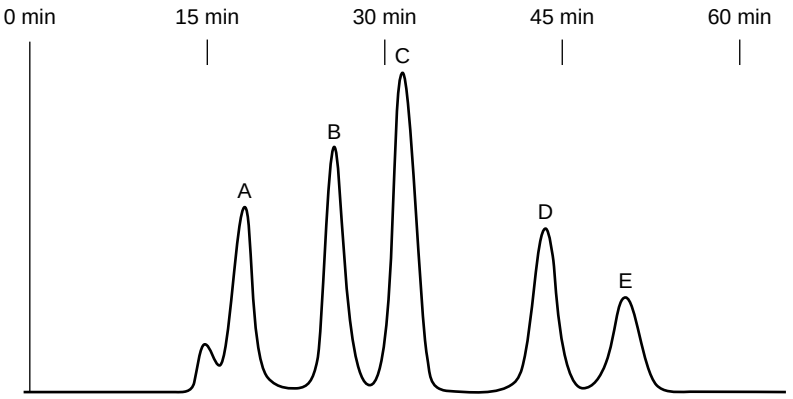


FIGURE 15.13 GPC chromatogram of calibration solution. Conditions: column, 700 × 25 mm with 70-g Bio-Beads SX-3, 490 mm bed length; mobile phase; methylene chloride; flowrate 5 mL/min; injection volume 5 mL; detector UV at 254 nm. Peaks: A—corn oil at 25 mg/mL; B—bis(2-ethylhexyl)phthalate at 1.0 mg/mL; C—methoxychlor at 0.2 mg/mL; D—perylene at 0.02 mg/mL; E—sulfur at 0.08 mg/mL (adapted from Reference 13.)

cleanup technique the organic extract is washed with either an alkaline or acidic aqueous solution to remove basic or acidic compounds from the extract. USEPA SW846 Method 3650 describes the acid–base partition cleanup (13). Consider an acidic compound (HA) that readily dissociates in solution to lose a proton and a basic compound (B) that readily accepts a proton in solution. The pH and the acid dissociation constants for each compound will determine the concentrations of each of the species shown in the following equations:

$$HA \leftrightarrow H^+ + A^- \tag{15.9}$$

$$B + H+ \leftrightarrow BH+ \tag{15.10}$$

If the pH is very high, A will be ionized and have a negative charge. On the other hand B will be neutral. If a liquid–liquid extraction is performed at this alkaline condition, A will remain in the aqueous phase and B will partition into the organic phase with any other neutral species that are present in solution. If A is an interference to B on the gas chromatogram it has been effectively been removed from B by performing a liquid–liquid extraction at basic conditions. The extract can be concentrated and B can be analyzed by GC without any inference from A. Suppose on the other hand, for example, that A is a chlorophenoxy herbicide and is the analyte of interest. Presently A is in the aqueous phase, but removed from B and other neutral organic compounds that have been extracted into the organic phase. The pH of the aqueous solution can be adjusted to a very low pH, and A will protonate (HA) and have a neutral charge. In this state a liquid–liquid extraction can be performed and A will partition into the organic phase. In effect A has been separated from any basic or neutral organic compounds that could pose interference. If the objective were to separate B from neutral organic compound, hydrocarbons for example, then the extractions could have been done in the reverse order. By extracting at low pH first, B is protonated (BH+) and remains in the aqueous phase and acidic and neutral compounds partition into the organic phase. Raising the pH and extracting then isolates B into the organic phase. The acid-base partition is a very elegant and simple strategy for isolating acidic and basic compounds and removing unwanted interferences.

15.9.3 Liquid–Solid Chromatographic Cleanups

Normal-phase liquid chromatography can be used to separate interfering compounds from SVOCs, pesticides, and PCBs. Three classical adsorbents—Florisil, alumina, and silicagel—are commonly used, although many other polar adsorbents are available. Table 15.7 shows some of the properties and characteristics of these adsorbents. Prior to the cleanup, the sample extract must be exchanged to a solvent that is compatible with the chromatographic separation. Because they are nonpolar, hexane and methylene chloride are common choices.

Cleanup may be accomplished using glass columns packed in the laboratory or using commercially available solid-phase cartridge containing these adsorbents.

TABLE 15.7 Properties of Common Adsorbents Used for Sample Cleanup

Alumina	Florisil	Silica Gel
Aluminum oxide Acidic (pH 4–5) Neutral (pH 6–8) Basic (pH 9–10)	Magnesium silicate, basic	Silicic acid, weakly acidic
400–450°C	650–675°C	150–160°C
Activity *I–V* 0–15% water	Deactivated with 3% water	Deactivated with <10% water
Method 3610B Method 3611C	Method 3620B	Method 3630C
Phthalate esters, nitrosamines, pesticides, aliphatic hydrocarbons, aromatic hydrocarbons	Organophosphorus pesticides, organochlorine pesticides, chlorinated hydrocarbons, phthalate esters, nitros amines, anilines, haloethers	PAHs, derivatized phenols, organochlorine pesticides, PCBs, aliphatic hydrocarbons, aromatic hydrocarbons
Basic and neutral SVOCs, 150 m^2/g	300 m^2/g	500 m^2/g

Source: Adapted from References 13 and 73.

The glass columns have a larger capacity to remove interferences from an extract than do the cartridges because more packing material is used. As a rule of thumb, one gram of adsorbent can remove 10–30 mg of interferences from a sample extract. The cartridges usually contain 1 or 2 g of an adsorbent while the columns often contain 10 or 20 g of adsorbent. A glass column may be 200–300 mm in length and have a diameter of 10–15 mm. The column is fitted with a stopcock to control the flow of liquid. Dry packing the adsorbent into the column with gentle tapping is a common practice, or the absorbent can be slurried with a solvent and poured into the column. The adsorbent is kept above the stopcock using a small plug of glass wool and a section of anhydrous sodium sulfate is placed at the head of the column to dry the extract and prevent water from reaching the adsorbent during the loading of sample extracts. The process is very labor-intensive since each sample requires a column. Solid-phase cartridges are available commercially and require less solvent although their capacity for cleanup is less. The US-EPA methods listed in Table 15.7 have procedures for both the larger glass columns and SPE. Polar compounds are retained on the adsorbents by polar interactions and can be eluted by increasingly polar solvents or mixtures of solvents. Nonpolar compounds show less retention on the sorbents listed in Table 15.7.

The Florisil cleanup of pesticides is an example of classical cleanup technique that has been used extensively for years. Prior to cleanup, the sample extract should be concentrated to 10 mL and be in hexane. A column containing 20 g of Florisil with a 2-cm layer of anhydrous sodium sulfate on top of the column is

prepared and rinsed with 60 mL of hexane. The 10-mL sample extract is loaded to the top of the column and eluted with 200 mL of a 6–94 volume/volume (v/v) mixture of ethyl ether–hexane. The process is repeated with 200 mL of 15–85 and 50–50 v/v ethyl ether–hexane. Most of the organochlorine pesticides and PCBs are found in the first fraction. By the end of the third fraction all have eluted. A total volume of 600 mL is required to elute all the organochlorine pesticides and PCBs. This volume of solvent needs to be concentrated to a final volume of 10 mL (usually). In the cartridge procedure one-gram Florisil cartridges are used. After rinsing with 4 mL of hexane, 1 mL of the sample extract is loaded onto the cartridge. The cartridge is eluted with 9 mL of 10–90 v/v of acetone: hexane. The PCBs and organochlorine pesticides are present in this fraction, and the extract can be evaporated to a final volume. The SPE vacuum manifold similar to the one pictured in Figure 15.9 is used for this cleanup. Many laboratories have switched from the column technique to SPE cleanups because of the savings in labor and solvent.

15.9.4 Miscellaneous Cleanups

Sulfur can be found in many environmental samples and causes extreme problems with some detectors, especially the electron-capture detector (ECD). Sulfur is often a problem in marine sediments. Orthorhombic sulfur (S_8) is a common form and is stable at ordinary temperatures although other rings and chains of sulfur are known to exist. The GPC cleanup will remove sulfur. In the past elemental mercury was added to pesticide and PCB extracts to reduce sulfur and form the insoluble mercuric sulfide. This technique has been abandoned because it is not safe. In USEPA SW846 Method 3660B, sulfur is removed from extracts using unoxidized copper powder or tetrabutylammonium sulfite. Concentrated sulfuric acid and potassium permanganate can be added to PCB extracts (USEPA SW846 Method 3665A) to destroy other organic compounds. Many of the pesticides and SVOCs are also oxidized by this treatment, therefore, only the PCBs can be determined on the extract after this cleanup step. (13).

15.10 DERIVATIZATION TECHNIQUES

A number of organic compounds cannot be separated by gas chromatography because they are nonvolatile. Others are difficult to separate because they have polar functional groups. Organic acids, such as the phenoxyacid herbicides and halogenated disinfectant byproducts, are examples of compounds that require derivatization in order to be perform good and productive gas chromatography. Phenols can be difficult to chromatograph and are sometimes derivatized. Another reason for chemical derivatization is to put a chemical label or tag on a compound so that is responsive to a particular detector. This is much more common in high-performance liquid chromatography (HPLC) where UV chromophores or fluorescent tags are used than in GC. Frequently derivatization techniques

replace the active hydrogen atom in functional groups of amines (–NH), carboxylic acids (–COOH), hydroxyls, (–OH), and thiols (–SH) using alkylation, silylation, or acylation. In alkylation the acid hydrogen on organic acids and phenols is replaced with an alkyl group, most often a methyl group. The more volatile and less active methyl ester is formed. Diazomethane is commonly used in many environmental methods to perform methylation. Silylation is the addition of trimethylsilyl ($-Si(CH_3)_3$) group to a molecule in replacement of the active hydrogen thus forming a more volatile compound. The compound, *N*,*O*-bis(trimethylsilylacetamide) (BSA), forms a highly stable derivative under mild reaction conditions. Acylation reagents target more highly polar functional groups such as amino acids or carbohydrates. Acylating agents can be obtained with a number of fluorinated configurations that can enhance detectability by an ECD. Hepafluorobutryric anhydride reacts with alcohols, amines, and phenols.

15.11 GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN ENVIRONMENTAL SAMPLES

In the following section gas chromatographic methods for the determination of VOCs in water and soil samples will be discussed. Most of the applications that will be presented are from USEPA methodology although some of the newer methods coming from the scientific literature will be discussed. The determination of VOCs in air will be discussed separately in Sections 15.16.1 and 15.16.2.

15.11.1 Analysis of Volatile Organic Compounds by GCMS

Often the USEPA SW846 Method 8260C (13) is required for the determination of VOCs in either water or soil samples. Because of the use of the mass spectrometer and the capillary column, a significant number of VOCs (e.g., target lists of 100 compounds are not uncommon) can be determined by this method in a single analytical run. A current application of this method for water samples is presented in the following discussion. Water samples are purged using helium onto a sorbent trap. After purging, the trap is heated and desorbed into the gas chromatograph for separation on a narrow-bore capillary column that is interfaced directly to a benchtop mass selective detector. Table 15.8 lists the conditions for the purge and trap and the GCMS for this application.

Five levels of calibration standards, ranging from 5 to 300 ng/mL are prepared in deionized water by diluting methanolic standard mixes containing the VOCs using syringes and volumetric flasks. Many laboratories purchase commercially available VOC mixes. Some compounds that are soluble in water and do not purge very well are calibrated at higher ranges. For example, isopropanol is normally calibrated from 80 to 1500 ng/mL. In this application prepared standards and samples, contained in 40-mL vials with Septa, are loaded into a refrigerated tray. During analysis an autosampler removes a 5-mL aliquot from each vial and transfers it to the purge vessel. During the transfer to the purge vessel, the surrogate

TABLE 15.8 Method Parameters for Determination of VOCs in Water by GC/MS

Purge–Trap Parameters	
Sample volume	5.0 mL
Trap temperature during purging	Ambient
Helium purge flow	40 mL/min
Sample temperature	40°C
Purge time	11 min
Trap	3- phase (Tenax/silicagel/charcoal)
Dry purge	None
Desorption temperature	185°C
Desorption time	4.0 min
Helium desorption flow	28 mL/min
Bake time/temperature	10 min at 180–220°C
Transfer line temperature	125°C
Gas Chromatograph Parameters	
Injector temperature	220°C
Split ratio	35:1
Column temperature	40°C for 5 min, ramp at 15°C/min to 200°C and hold for 5 min
Column	DB-624, 30-m × 0.25-mm-i.d. × 1.4-μm film
Helium carrier flow (EPC control)	0.8 mL/min
Transfer line temperature to MSD	250°C
MSD Parameters	
MSD interface	Direct
Ionization	Electron impact (EI) at 70 eV
Tune	USEPA 1,4-bromofluorobenzene
Scan range	35–300 amu (positive ions)
Scan speed	2–3 scans/s

standards (1,2-chloroethane-d_4, toluene-d_8, bromofluorobenzene) and the internal standards (bromochloromethane, 1,4-difluorobenzene, and chlorobenzene-d_5) are added. Some of these compounds contain deuterium, which alters their molecular weights and differentiates them from the common target compounds. The total-ion chromatogram (TIC) of a 50-ng/mL aqueous standard, which contains approximately 60 VOCs, including internal standards, surrogate standards, and target compounds, is shown in Figure 15.14.

The chromatogram in Figure 15.14 has less than the usual number of compounds that are routinely determined by this method, but was presented so that the graphics could be legible. The large peak at the beginning of the TIC is from carbon dioxide. A high split ratio was used (35–1), therefore if desired, method sensitivity could be improved by lowering the spilt ratio. An internal standard calibration is performed. Target compounds in samples are identified by their retention time and their mass spectrum. Quantification is usually based on the area of the most prevalent extracted ion (m/z) from the spectrum of the

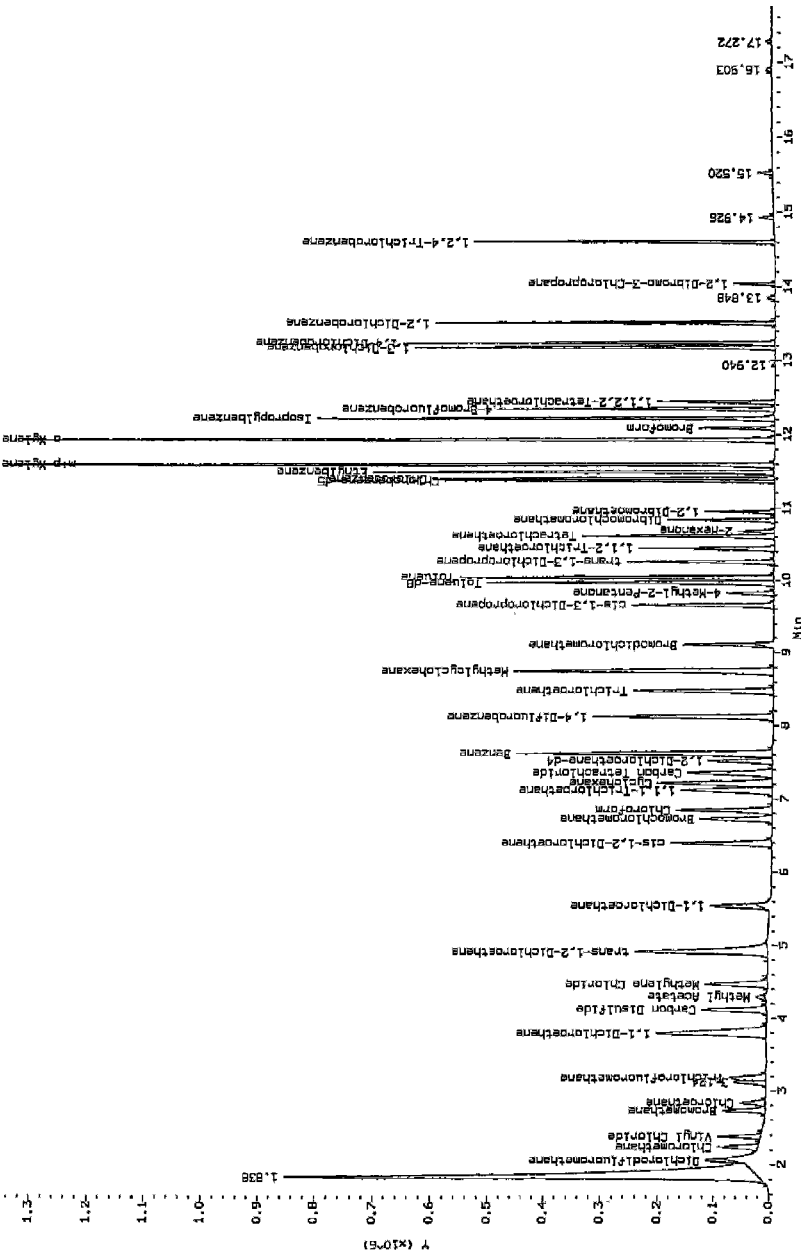


FIGURE 15.14 Total-ion chromatogram of an aqueous VOC standard at 50 ng/mL by GCMS USEPA Method 8260B; see Table 15.8 for instrument conditions (courtesy of Lancaster Laboratories, GC/MS VOA Group).

target compound known as the "quantitation or quant ion." Another advantage of the mass spectrometer as a detector is that unknown peaks can be identified by performing a library search. The spectrum of an unknown peak is searched against a spectral database of electron-impact (EI) ionization spectrum to find the best match or matches and to tentatively identify the unknown compound. The present National Institute of Science and Technology (NIST) database contains the EI spectra for approximately 120,000 organic compounds.

A number of quality control restrictions are placed on the method. The mass spectrometer must be checked every 12 h with 1,4-bromofluorobenzene (BFB) to ensure that its spectrum meets the tuning criteria established in the method. A method blank must be generated using laboratory reagent water (ASTM type II) every 12 hours to demonstrate the analytical system is free of contamination. The average relative response factor (RRF) for each target compound from the five calibration standards is normally used for quantification. If, however, the relative standard deviation (RSD) of response factors from the five calibration standards for a target analyte is greater than 15%, then a linear curve must be generated for that target compound. A sample is spiked in duplicate, the matrix spike (MS) and the matrix spike duplicate (MSD), and the recoveries of target list compounds must fall within established limits. A laboratory control sample (LCS), which is laboratory reagent water spiked with the target analytes, is also analyzed. The reporting limit for most of the target compound using this method is 1 ng/mL or 1 ppb in water.

Soils or other solids can be extracted with methanol and an aliquot of the methanol added to deionized water and determined by the method described above. Because the detection limit is higher these are referred to as *high-level soils*. Low soils are mixed with sodium sulfite and water and purged directly. It is preferable to mix the sodium sulfite and weigh the soil in the field so that the vial containing the sample does not a have to opened in the laboratory. Basically the same instrumental conditions are used to detect the VOCs in soils. USEPA Method 524.1 (14) is used for determining VOCs in drinking water. Because lower sensitivity is desired, a 25-mL aliquot of water is sometimes purged and the calibration range is lower. Similar instrumental conditions for the GCMS, however, would be used. A different set of internal standards and surrogate standards are also used for the drinking water method.

15.11.2 Determination of Aromatic and Halogenated Volatile Organic Compounds Using Photoionization and Electrolytic Conductivity Detectors

The mass spectrometer is a universal detector that can be used for nearly every organic compound that can pass through a gas chromatograph. It is not used universally for two main reasons—some laboratories cannot afford to purchase a GCMS, and sometimes extremely low detection limits beyond that of the mass spectrometer are required. In the next application a method for the simultaneous determination of aromatic and halogenated VOCs will be discussed. The method

[USEPA SW846 Method 8021B (13)] requires that a photoionization detector (PID) and an electrolytic conductivity detector (ELCD) or Hall detector be connected in series. The PID is a nondestructive detector and is first in line after the column to detect the aromatic VOCs. The effluent from the PID flows into ELCD for detection of the halogenated VOCs. Two chromatograms are produced, one for the PID and one for the Hall detector. Sometimes the situation becomes more complicated and a second confirmation column is required. The confirmation column must have a different phase than the primary column. Often times the flow coming from desorbing the trap is split between these two columns using a "Y" connection. Two separations are occurring simultaneously in the oven of the chromatograph with each going to a set of detectors. A total of four chromatograms result. Table 15.9 lists the instrumental conditions for the separation of the aromatic and halogenated VOCs. Only one column has been presented in this application.

The PID is calibrated from aqueous standards prepared from methanolic mixes of the VOCs as described above. Six levels of standards are prepared ranging

TABLE 15.9 Gas Chromatographic Conditions for Determination of Aromatic and Halogenated VOCs

Purge–Trap Parameters	
Sample volume	5.0 mL
Trap temperature during purging	Ambient
Helium purge flow	40 mL/min
Sample temperature	Ambient
Purge time	11 min
Trap	3- phase (Tenax/silicagel/charcoal)
Dry purge	None
Desorption temperature	180°C
Desorption time	4.0 min
Helium desorption flow	28 mL/min
Bake time/temperature	10 min, at 180–200°C
Transfer line temperature	125°C
Gas Chromatograph Parameters	
Injector temperature	250°C
Column temperature	30°C for 1 min; ramped at 5°C/min to 60°C and hold for 1 min; ramped at 19°C/min to 200°C and hold for 4.7 min; ramped at 30°C/min to 250°C and held for 6.0 min
Column	J&W, VRX 75-m × 0.45-mm × 2.55- μm film
Helium carrier flow	10 mL/min
Makeup gas to PID	20 mL/min
PID temperature	250°C
Hydrogen to ELCD	120 mL/min
Temperature-Ni reaction tube	900°C
Flow 1-propanol	60 μL/min

from 0.5 to 140 μg/L. An internal standard calibration (see Chapter 8) is used and based on fluorobenzene. The ELCD is calibrated from 0.5 to 100 μg/L using an external standard calibration. Quantification is based on the peak height rather than peak area. A common surrogate standard, 1-bromo-4-chlorobenzene, for both the PID and ELCD is added to all aqueous samples prior to purging. Standards and samples are placed in 40-mL vials that are placed in a cooled compartment of an autosampler until analysis. The autosampler delivers a 5-mL aliquot the sample to the purge vessel for purging. Most soils are first extracted with methanol, and an aliquot of the methanol is added to water for purging. Example chromatograms of a midlevel standard (each compound at approximately 50 μg/L) from the PID and the ELCD are shown in Figure 15.15.

Compounds that contain halogens and are aromatic display response on both (e.g., chlorobenzene) detectors. Compounds that have a double bond, such as vinyl chloride, are ionized on the PID (11.7 keV), but show a much weaker response than on the Hall detector. Common quality control samples such as a method blank, a matrix spike and a matrix spike duplicate, and a laboratory control sample are required when analyzing samples by this method. A continuing calibration or check standard is injected every 12 h to verify the calibration.

15.11.3 Methods for Determining Gasoline-Range Organics

A large effort in environmental analysis involves monitoring fuel spills that occur or supporting the remedial action that environmental engineering firms use to clean up these spills. Fuels are often released into the environment through leaking underground storage tanks (LUSTs). The release of gasoline is common. Once in the soil the aromatic components of the gasoline migrate more quickly and often find their way into the groundwater. The aromatic compounds, benzene, toluene, ethylbenzene, and the xylene isomers (meta, para, and ortho), known as BTEX are often of primary concern because they pose the greatest threat to human health.

Gasoline is a petroleum distillate that contains of a wide range of light hydrocarbons. The American Petroleum Institute defines this range as all components that elute between 2-methylpentane and 1,2,4-trimethylbenzene or from approximately C6 to C10. Other sources may expand this range to C5–C12 to truly reflect the gasoline pattern seen on a gas chromatogram. A variety of methods and approaches exist for the determination of gasoline-range organics (GRO) in water or soil samples. Many of the states have their own methods and approach the problem differently. Purge and trap is used most often used to extract GRO from water and soil samples and to obtain low detection limits. The flame ionization detector (FID) is commonly used to detect GRO. Sometimes a PID is used in series before the FID to detect the aromatic hydrocarbons. In some applications the flow from the column will be split between the two detectors. USEPA Method 8015B (13) requires only the FID. Calibration and quantification can be based on single-component standards or can be the sum of the peak areas over the GRO range. In the latter approach, a source of gasoline is used to prepare standards.

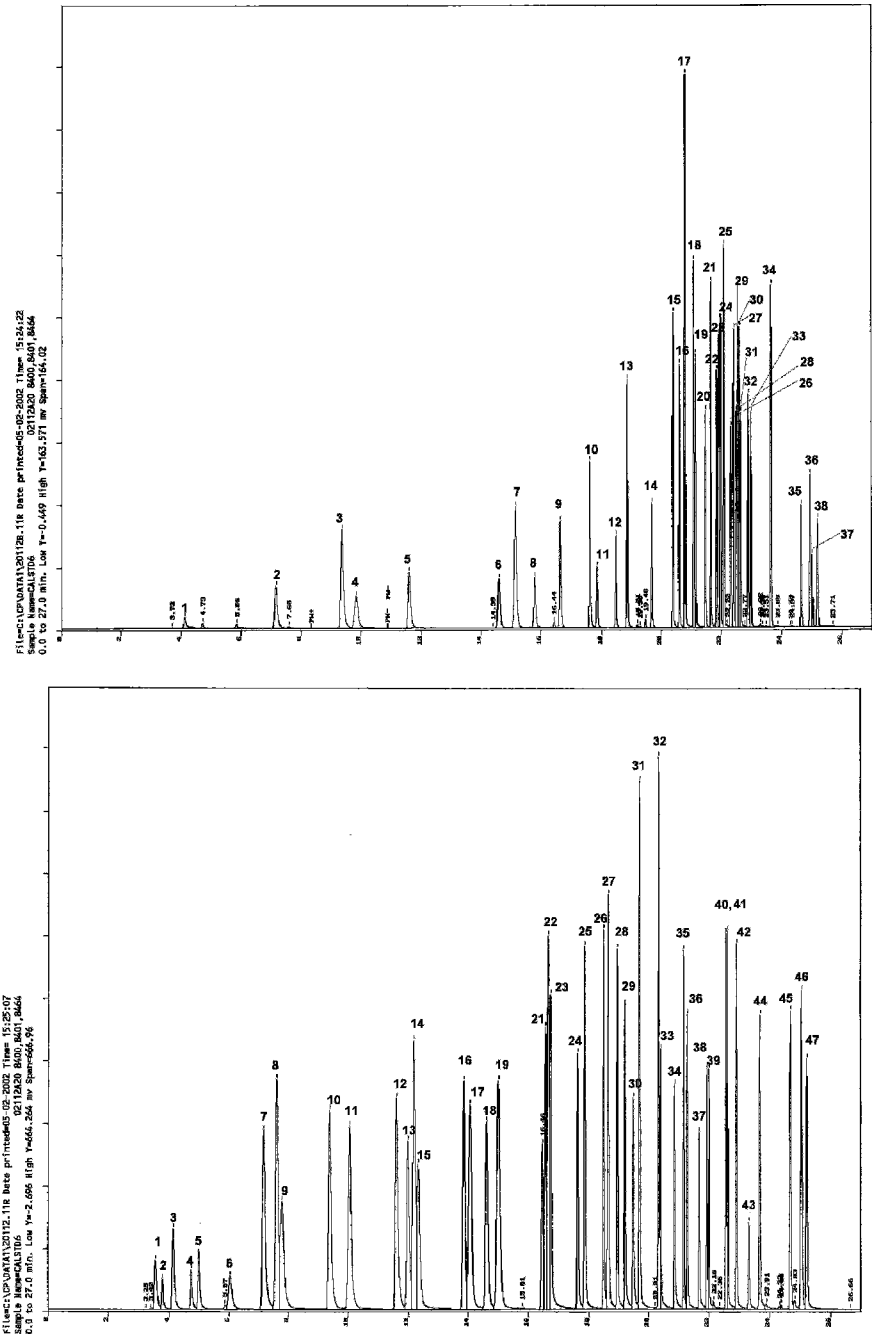


FIGURE 15.15 Chromatograms of aromatic and halogenated VOCs in water by purge–trap method using the PID (top) and ELCD (bottom); see Table 15.9 for instrumental conditions (courtesy of Lancaster Laboratories).

PID Detector

	RT (min)	Compound
1.	4.14	vinyl chloride
2.	7.17	1, 1-dichloroethene
3.	9.38	trans-1, 2-dichloroethene
4.	9.84	methyl-t-butyl ether
5.	11.61	cis-1, 2-dichloroethene
6.	14.61	1,1-dichlopropene
7.	15.16	benzene
8.	15.80	fluorobenze(IS)
9.	16.65	trichloroethene
10.	17.62	2-chloroethyl ether
11.	17.86	cis-1, 3-dichloropropene
12.	18.49	trans-1, 3-dichloropropene
13.	18.88	toluene
14.	19.70	tetrachloroethene
15.	20.40	chlorobenzene
16.	20.62	ethylbenzene
17.	20.81	m, p-xylene
18.	21.10	styrene
19.	21.16	o-xylene
20.	21.49	isopropylbenzene
21.	21.68	bromobenzene
22.	21.86	n-propylbenzene
23.	21.92	2-chlorotoluene
24.	22.00	4-chlorotoluene
25.	22.11	1, 3, 5, -trimethylbenzene
26.	22.34	tert-butylbenzene
27.	22.43	1, 2, 4, -trimethylbenzene
28.	22.51	Sec-butylbenzene
29.	22.56	1, 3-dichlorobenzene
30.	22.62	1, 4-dichlorobenzene
31.	22.67	p-isopropyltoluene
32.	22.91	1, 2-dichlorobenzene
33.	23.00	n-butylbenzene
34.	23.68	bromo-4-chlorobenzene SUR)
35.	24.68	1, 2, 4 –trichlorobenzene
36.	24.97	Naphthalene
37.	25.04	hexachlorobutadiene
38	25.22	1, 2, 3 –trichlorobenzene

Hall Detector

	RT (min)	Compound
1.	3.56	dichlorodifluoromethane
2.	3.80	chloromethane
3.	4.16	vinyl chloride
4.	4.75	bromomethane
5.	5.00	chloroethane
6.	6.04	triclorofluoromethane
7.	7.18	1, 1-dichloroethene
8.	7.61	methylene chloride
9.	7.80	trichlorotrifluoroethane
10.	9.39	trans-1, 2-dichloroethene
11.	10.06	1, 1-dichloroethane
12.	11.62	cis-1, 2-dichloroethene
13.	12.00	bromochloromethane
14.	12.20	chloroform
15.	12.34	2, 2-dichloropropane
16.	13.87	1, 2-dichloroethane
17.	14.07	1, 1, 1-trichloroethane
18.	14.62	1, 1-dichloropropene
19.	15.07	carbon tetrachloride
20.	16.46	dibromomethane
21.	16.57	1, 2-dichloropropane
22.	16.67	trichloroethene
23.	16.74	bromodichloromethane
24.	17.64	2-chloroethyl vinyl ether
25.	17.87	cis-1, 3-dichloropropene
26.	18.50	trans-1, 3-dichloropropene
27.	18.66	1, 1, 2-trichloroethane
28.	18.96	1, 3 ñdichloropropane
29.	19.22	dibromochloromethane
30.	19.50	dibromoethane
31.	19.71	tetrachloroethene
32.	20.34	1, 1, 1, 2-Tetrachloroethane
33.	20.42	chlorobenzene
34.	20.87	bromoform
35.	21.17	1, 1, 2, 2-Tetrachloroethane
36.	21.30	1,2,3-trichloropropane
37.	21.69	bromobenzene
38.	21.94	2-chlorotoluene
39.	22.01	4-chlorotoluene
40.	22.58	1, 3-dichlorobenzene
41.	22.63	1, 4-dichlorobenzene
42.	22.93	1, 2-dichlorobenzene
43.	23.34	1, 2-dibromo-3-chloropropane
44.	23.69	bromo-4-chlorobenzene (SUR)
45.	24.70	1, 2, 4 –trichlorobenzene
46.	25.06	hexachlorobutadiene
47.	25.23	1, 2, 3 –trichlorobenzene

FIGURE 15.15 (*Continued*)

Since brands of gasoline are not uniform, Method 8015B recommends that the gasoline from the site of the spill (e.g., the gasoline remaining in a tank suspected of leaking) accompany the samples and be used for calibration. Weathering, which occurs over time when gasoline is released into the environment, further complicates the process of identification and quantification. The more volatile fraction of gasoline will most likely have evaporated unless sampling has occurred soon

after the spill, leaving only the heavier fraction of gasoline to appear in the chromatograms of soil and water samples taken from the site. The gasoline pattern seen in the chromatogram may also be influenced by the solubility of the different components in the gasoline, which migrate at different rates through the soil.

In the following example, a soil sample (5 g) was spiked with approximately 1 mg/kg of gasoline, and extracted with 5 mL of methanol. A 100-μL aliquot of the extract was added to water and purged onto a Tenax trap. The trap was desorbed onto a megabore capillary column for the separation and detection was accomplished by a PID and FID in series. The instrument conditions for this separation are listed in Table 15.10.

The chromatograms of the soil sample spiked with gasoline are shown in Figure 15.16.

In this example the chromatogram from the FID was integrated and the area summed from 7.07 to 18.48 min, which corresponds to the range from methyl pentane to 1,2,4-trimethylbenzene, to determine GRO. Trifluorotoluene (TFT) was added as a surrogate standard at 30 ppb to the soil prior to extraction, and its area was subtracted from the total GRO area during quantification. The rules that are applied to the integration range and the standard that is used for determining GRO will greatly influence the amount of GRO that is reported for regulatory purposes. In this example, it is obvious that the gasoline pattern

TABLE 15.10 Instrumental Condition for Purge and Trap GC/PID/FID for the Determination of GRO in Soil and Water

Purge–Trap Parameters	
Sample volume	5.0 mL
Trap temperature during purging	Ambient
Helium purge flow	40 mL/min
Sample temperature	Ambient
Purge time	11 min
Trap	Tenax
Dry purge	6 min
Desorption temperature	180°C
Desorption time	4.0 min
Helium desorption flow	28 mL/min
Bake time/temperature	9 min at 180°C
Transfer line temperature	150°C
Gas Chromatograph Parameters	
Injector temperature	220°C
Column temperature	35°C for 2 min; ramped at 10°C/min to 75°C; ramped at 6°C/min to 125°C; ramped at 20°C/min to 250°C and held for 7 min
Column	Restek RTx-502.2, 75-m × 0.45-mm × 2.55- μm film
Helium carrier flow	4.5 mL/min
PID and FID temperatures	265°C

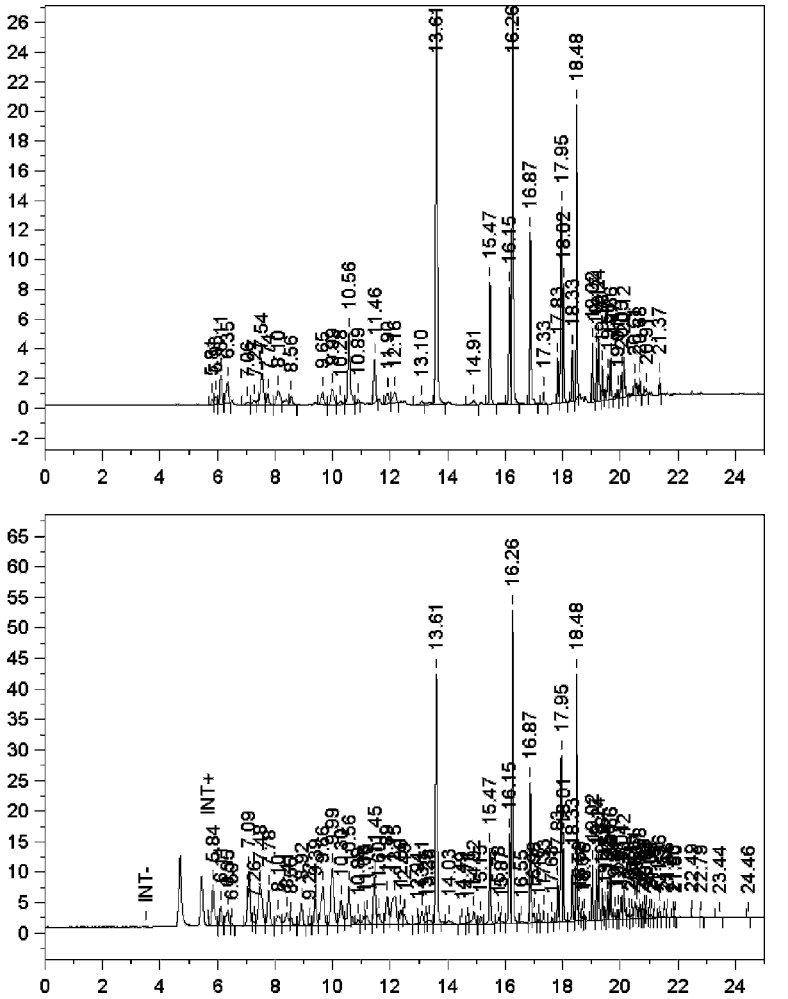


FIGURE 15.16 PID chromatogram (top) and FID chromatogram (bottom) of a soil sample spiked with 1 mg/kg of gasoline; instrumental conditions listed in Table 15.10. GRO integration from 7.07 to 18.48 min—2-methylpentane to 1, 2, 4-trimethylbenzene. PID chromatogram—MTBE (7.54 min), benzene (10.56 min), trifluorotoluene—surrogate standard. (11.46 min), toluene (13.61), ethyl benzene (16.15 min), *m*,*p*-xylenes (16.26 min), *o*-xylene (16.88 min). (Courtesy of Lancaster Laboratories).

extends beyond the 1,2,4-trimethylbenzene peak, but this area is not included during quantification. The BTEX compounds are determined individually using the PID. Methyl-tertiary butyl ether (MTBE) and tertiary butyl alcohol (TBA), two oxygenates in gasoline and of environmental concern, are often monitored using GRO methods.

15.11.4 Alternative Methods for Determining Volatile Compounds

The majority of the USEPA methods require the use of purge of trap. The technique of purge and trap has a number of limitations, however. Carryover or cross-contamination of samples can occur in the purge and trap after introducing samples containing high levels of VOCs. The long cycle time and bake times associated with the purge and trap can be detrimental to increased productivity through shortened runtimes and fast gas chromatography. It would be advantageous for the sake of analysis speed and to reduce memory effects, to remove the purge and trap from VOC analysis. Injecting a larger static headspace volume is one option. Hino et al. described a method for sampling the entire static headspace of a vial (approximately 9 mL above 5-mL sample) to increase sensitivity (74). A steam of helium was used to flush the headspace onto a small Tenax trap cooled with carbon dioxide for approximately 15 s. After trapping, the light gases were thermally desorbed into a GCMS for subsequent analysis (74). Sacks and others described a device for the high-speed gas extraction of VOCs from aqueous samples (75). Using elevated temperature with a reflux device to manage water and prevent analyte loss, quantitative recoveries were achieved in 30 s for benzene. The device was interfaced to a cryofocusing inlet system for high-speed gas chromatography (75). In another approach, Current and Borgerding used high-speed gaseous extraction to introduce VOCs into a cryotrap for subsequent high-speed chromatography (76). Samples of 100 μL were quantitatively extracted in approximately 20 s by a 200-mL/min stream of nitrogen in a 2.5-mL spray chamber (76). The use of static headspace sampling and solid-phase microextraction (HSSPME) has been another approach to eliminating the purge and trap. A host of applications can be found using HSSPME for determining VOCs of environmental relevance (7,77). A few specific references are highlighted in Table 15.11.

15.12 GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF SEMIVOLATILE ORGANIC COMPOUNDS

In the analysis of semivolatile organic compounds the mass spectrometer is again the detector of choice, because of the increased confidence in peak identification

TABLE 15.11 Applications of SPME to VOCs in Environmental Analysis

Analyte	Method	References
Water-soluble VOCs	Solution and HSGC/MS	54,78,79
Formaldehyde	HSGC/FID after derivatization	80
170 VOCs in fish tissue	HSGC/MS	81
Fuel-related hydrocarbons	GCFID	82,83
71 VOCs	Solution and HSGC/FID	84,85
Ethanol, MTBE, and related oxygenates	Solution and HSGC/MS	86,87
Trimethylamine, propionic and butyric acids, and sulfur compounds	HSGC/FID	88
Trifluoroacetic acid	HSGC/FID after derivatization	89

and its ability to search and identify unknowns. Other detectors are used for specific classes of SVOCs, but usually are chosen because they are less expensive or in some instances, to obtain lower detection limits.

15.12.1 The Determination of Semivolatile Organic Compounds by Gas Chromatography and Mass Spectrometry

USEPA Method 8270C (13) is probably the most used method for the determination of SVOCs in soil and water samples. Water samples are first extracted with methylene chloride at high pH (>11) using a liquid–liquid or continuous liquid extraction. The pH is then adjusted to <2 and the extraction is repeated. The two fractions, acid extractable and the base neutral, are usually combined for the solvent evaporation step. If a cleanup is to be used, GPC is usually chosen, because of the large number of compounds that are usually monitored. Soil samples are extracted after drying with sodium sulfate using 1–1 acetone–methylene chloride and the sonication or Soxhlet extraction. Thirty grams of soil and one liter of water are normally extracted and concentrated to a final volume of 1.0 mL to obtain the required detection limits. The six surrogate standards, which are added to the soil or water prior to extraction, are 2-fluorophenol, 2,4,6-tribromophenol, phenol-d_6, 2-fluorobiphenyl, nitrobenzene-d_5, and terphenyl-d_{14}. Prior to injection six internal standards are added to the 1-mL extract. These include naphthalene-d_8, 1,4-dichlorobenzene-d_4, acenaphthene-d_{10}, phenathrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}.

Normally a splitless injection of 1 or 2 μL is made onto a standard (DB-5, 30 m × 0.25-mm × 1.0- μm film) capillary column. A split time ranging from 30 s to 1 min is normal and depends on the brand of the gas chromatograph. The column is slightly polar and contains 5%diphenyl–95% dimethylpolysiloxane as the stationary phase. Columns with thinner films (e.g., 0.25 μm) are sometimes chosen because of their low bleed characteristics and because they clean up more readily during baking. In some cases the thinner film can also provide increased resolution; however, thinner films have lower capacity than do thicker films and the range of the calibration may require abbreviation. Often a 5-m length of fused-silica guard column is placed prior to the analytical column to protect it from nonvolatile impurities and to aid in solvent-focusing the analytes. The helium carrier gas is usually maintained at about 1.0 mL/minute to stay within the pumping limits of the MSD. Mass spectrometers with greater pumping capacity allow for greater column flow and permit the use of capillary columns with larger diameters (e.g., 0.32 mm). The oven temperature is programmed from about 40 to 300°C using a temperature ramp of approximately 10°C/min. At the end of the run the oven is often held at the elevated temperature to bake off contaminates and prepare the column for the next injection. Resolution must be sufficient to separate structural isomers. Benzo(*b*)fluoranthene and benzo(*k*)fluoranthene are examples of a critical pair that must be separated. Method 8270C requires that the valley between the two isomeric peaks be less than 25% of the sum of the two peak heights. Of course, each environmental laboratory and sometimes each

analyst claim to have the perfect temperature ramp and column to achieve the optimum separation. In reality a truly optimized separation is nearly impossible because of the large number of compounds that are often separated. Separation times of 50–60 min are common using this method.

The MSD is tuned to meet the USEPA tuning criteria established in the method for 50 ng of DFTPP (decafluorotriphenylphosphine) and must be demonstrated every 12 h. The harsh reality is that this tuning criterion was set in 1975 (90) and mass spectrometers have changed greatly since that time. Many modern instruments must sometimes be detuned to meet this criterion, yet the USEPA has maintained this standard. Included in the DFTPP tuning solution are DDT and endrin to check the column and injection port inertness. Endrin decomposes to endrin aldehyde and endrin ketone, while DDT breaks down to DDE and DDD. Breakdown should not exceed 20% for these compounds. Benzidine and pentachlorophenol are often added to this solution to demonstrate that the gas chromatographic system does not have active sites that will cause peak tailing. The MSD is scanned from 35 to 500 amu for SVOCs and at a rate of approximately 1 scan/s, although faster scan rates are possible on modern instruments.

An internal standard calibration with at least five levels is performed over the range of 5 μg/mL to approximately 120 μg/mL. Standards are prepared in methylene chloride. Because of the long list of compounds, laboratories often purchase commercially available mixes and dilute these to prepare the calibration standards. Quantification is based on the area of the primary quantitation ion (m/z) for each target compound and the average relative response factor of the calibration standards. The RSD of the relative response factors for each target compound must be less than 20%. A method blank, matrix spikes (MS and MSD), and the laboratory control sample (LCS) must accompany each batch of samples. Target compounds are identified by their retention time and their mass spectrum. Most compounds are reported down to 1 μg/L in water samples and 330 ppb in soil samples.

The USEPA GCMS method for the determination of SVOCs in drinking water is Method 525.2 (91). Target compounds, surrogate standards, and internal standards are extracted by passing one liter of water over a 47-mm Empore disk having a C18 solid phase. The disk is eluted with small volumes of ethyl acetate and methylene chloride, which after drying with sodium sulfate, are evaporated to a final volume of 1 mL. The GCMS conditions for this method are shown in Table 15.12.

An example total-ion chromatogram of a water sample that has been spiked with the target compounds (matrix spike) at approximately 2 μg/L is shown in Figure 15.17.

The internal standards that are used are acenaphthene-d_{10}, chrysene-d_{12}, and phenanthrene-d_{10}. The three surrogate standards are 1,3-dimethyl-2-nitrobenzene, triphenylphosphate, and perylene-d_{12}. Lower sensitivity is the goal in this method, and, therefore, six calibration standards in ethyl acetate over the range from 0.1 to 10 μg/L are injected. A longer splitless time (2 min), which increases the amount of analyte loaded unto the column, and the thinner-film column

TABLE 15.12 GC/MS Conditions for Determination of SVOCs in Drinking Water by USEPA Method 525.2

Gas Chromatographic Parameters	
Injector temperature	275°C
Injection volume	2 μL
Splitless	2 min
Column temperature	45°C for 2 min, ramp at 15°C/min to 150°C; ramp at 10°C/min to 300°C and hold for 4 min
Column	Rtx-5, 30-m × 0.25-mm × 0.25-μm film
Helium carrier flow (EPC control)	1.0 mL/min
Transfer line temperature to MSD	300°C
MSD Parameters	
MSD interface	Direct
Tune	USEPA DFTPP (5 ng)
Ionization	EI at 70 eV
Scan range	45–450 amu (positive ions)
Scan speed	1.5 scans/s

(0.25 mm i.d.) contribute to lower detection limits. The method detection limits for many of the compounds given in Method 525.2 are less than 100 ng/L (parts per trillion).

15.12.2 Semivolatile Organic Compounds Determined Using Alternative Detectors

A number of USEPA methods exist for the determination of classes of SVOCs in environmental samples using detectors other than the mass spectrometry. Some of these methods are discussed in the following sections.

15.12.2.1 Polynuclear Aromatic Hydrocarbons

USEPA SW846 Method 8310 is a popular HPLC method for determining PAHs using the fluorescence and UV detectors (13), but is beyond the scope of this text. USEPA Method 8100 lists 24 PAHs that can be determined by GCFID following an appropriate extraction (13). The method lists a packed column (1.8 m × 2 mm i.d. packed with 3% OV-17 on Chromosorb w-AW-DCMS, 100/120 mesh) or a 30-m narrow-bore capillary column with a SE-54 phase. A silicagel cleanup is recommended and will most likely be needed for dirty environmental samples. PAHs can be detected by the FID, but in complex samples this approach is usually unsatisfactory. The mass spectrometer is much more selective to the PAHs. Because of the aromatic character of the PAHs, a strong molecular ion is usually observed. The spectra are easily recognized and strong quantification ions are available (92). Often the mass spectrometer is operated using selective-ion monitoring (SIM) to further increase sensitivity and selectivity to the PAHs.

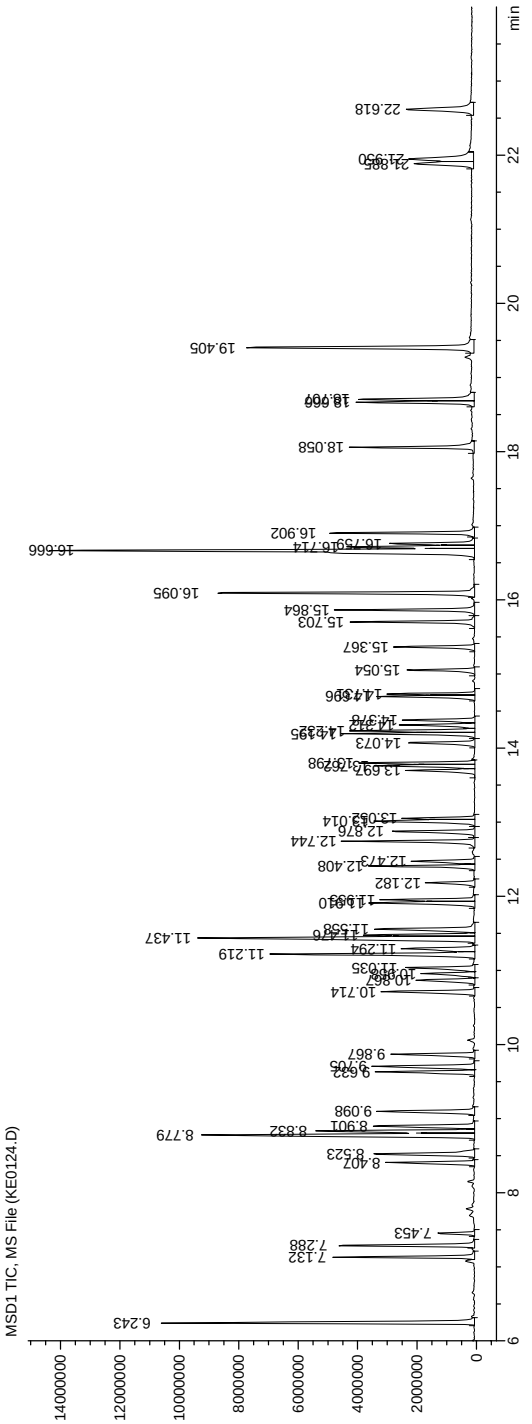


FIGURE 15.17 Total-ion chromatogram of matrix spike generated using USEPA Method 525.2; instrumental conditions presented in Table 15.12 (courtesy of Lancaster Laboratories).

Internal Standards		
Acenaphthene-d10	**8.78**	**164**
Phenanthrene-d10	**11.44**	**188**
Chyrsene-d12	**16.67**	**240**
Target Compounds		
Naphthalene	6.26	128
2-Methylnaphthalene	7.13	142
1-Methylnaphthalene	7.28	142
Hexachlorocyclopentadien	7.46	237
Dimethylphthalate	8.41	163
Acenaphthylene	8.53	152
Acenaphthene	8.84	153
2-Chlorobiphenyl	8.90	188
Dibenzofuran	9.10	168
Diethylphthalate	9.63	149
Fluorene	9.71	66
Propachlor	9.87	120
2,3-Dichlorobiphenyl	10.71	222
Hexachlorobenzene	10.88	284
Simazine	10.96	186
Atrazine	11.04	215
Pentachlorophenol	11.22	266
Lindane	11.29	183
Phenanthrene	11.47	178
Anthracene	11.56	178
2,4,5-Trichlorobiphenyl	11.96	256
2,2',4,4'-Tetrachlorobiphe	12.88	292
SAN Trimer	12.87	129
Alachlor	12.41	160
Heptachlor	12.47	100
Metribuzin	12.18	198
Di-n-butylphthalate	12.74	149
Metolachlor	13.02	162

Surrogate Standards		
2-NMX	**6.24**	**134**
Triphenyylphosphate	**16.10**	**326**
Perylene-d12	**19.40**	**264**
Target Compounds		
Aldrin	13.05	66
Heptchlor epoxide	13.70	81
Pentachlorobiphenyl	13.76	326
Fluoranthene	13.80	202
gamma-Chlordane	14.08	375
Butachlor	14.20	176
Pyrene	14.23	202
alpha-Chlordane	14.31	375
trans-Nonachlor	14.39	409
Dieldrin	14.69	79
Hexachlorobiphenyl	14.74	360
Endrin	15.06	81
cis-Nonachlor	15.37	409
Butylbenzylphthalate	15.70	149
di(2-Ethylhexyl)adipate	15.86	129
Benzo[a]anthracene	16.63	228
Heptachlorobiphenyl	16.67	394
Methoxychlor	16.66	227
Chyrsene	16.72	228
Octachlorobiphenyl	16.76	430
di(2-Ethylhexyl)phthalate	16.90	149
Di-n-octylphthalate	18.06	149
Benzo[b]fluoranthene	18.67	252
Benzo[k]fluoranthene	18.71	252
Benzo[a]pyrene	19.40	252
Indeno[1,2,3-cd]pyrene	21.89	276
Dibenz[a,h]anthracene	21.95	278
Benzo[g,h,i]perylene	22.62	276

FIGURE 15.17 (*Continued*)

15.12.2.2 Haloethers and Chlorinated Hydrocarbons

USEPA SW846 Methods 8111 and 8121 describe dual-column techniques for the gas chromatographic determination of haloethers and chlorinated hydrocarbons, respectively (13). Soils and waters can be extracted by any of the USEPA methods described above and the extracts changed over to hexane. Sample extracts may be cleaned up using GPC or Florisil SPE. A single megabore capillary column or two megabore columns connected in parallel by a "Y" configuration soon after the injection port, are used for the separation. The electron-capture detector (ECD) is used for both methods. In Method 8111, 24 haloethers are listed. The megabore columns that are recommended for separating the haloethers are as follows:

Column 1—DB-5 (5% diphenyl–95% dimethyl polysiloxane phase), 30 m × 0.53 mm × 0.83 or 1.5-μm film.

Column 2—DB-1701 (14% cyanopropyl–95% dimethyl polysiloxane phase), 30 m × 0.53 mm × 1.0-μm film

In Method 8121, 22 chlorinated hydrocarbons are listed. The columns recommended for their separation are listed below:

Column 1—DB-210 (trifluoromethyl silicone), 30 m × 0.53 mm × 1.0-μm film
Column 2—DB-Wax (polyethylene glycol), 30 m × 0.53 mm × 1.0-μm film

The two columns listed for Method 8111 can also be used for the chlorinated hydrocarbons. Compounds are separated using temperature ramps in both methods. Helium is listed as the carrier gas with flows of 5–10 mL/min recommended for each column. Sub-ppb detection limits are possible with the ECD. Target compounds are identified by their peak retention times. If two columns are used, this identification can be confirmed by the retention times on the second column. When qualitative identification of the haloethers and the chlorinated hydrocarbon target compounds is made, the method recommends GCMS confirmation.

15.12.2.3 Phthalate Esters

The phthalate esters can also be determined by GCECD after an appropriate solvent extraction from aqueous or solid samples. USEPA Method 8061 lists 16 phthalate esters (13). Solid-phase extraction using C18 membrane disks can be used for aqueous samples, but the pH must be maintained between 5 and 7 to prevent hydrolysis of the phthalate esters. Solids or soils can be extracted by sonication or Soxhlet extraction using 1–1 methylene chloride–acetone. GPC or the Florisil cleanup may be necessary. Extreme care must be taken not to contaminate samples with phthalate esters that are ubiquitous in the laboratory. The solvent needs to be exchanged to hexane. Two megabore capillary columns that are connected by a "Y" in parallel are recommended for separation. Detection is accomplished by dual ECDs. The conditions for the GC are listed in Table 15.13.

Diphenylphthalate, diphenylisophthalate, and dibenzylphthalate are the surrogate standards that are added at 50 μg/L to water samples and at 830 μg/Kg to soils prior to extraction. An internal standard or external standard calibration can

TABLE 15.13 Gas Chromatographic Conditions for USEPA Method 8061, Phthalate Esters

Injector temperature	250°C
Injection volume	2 μL
Helium carrier flow	6 mL/min
Column 1: DB-5, 30-m × 0.53-mm × 1.5-μm film	
Column 2: DB-1701, 30-m × 0.53-mm × 1.0-μm film	
Oven temperature: 150°C for 0.5 min, ramp to 220°C at 5°C/min, ramp to 275°C at 3°C/min and hold for 13 min	
ECD temperature	320°C
ECD makeup (nitrogen)	19 mL/min

Source: Taken from Method 8061A, Reference 13.

be used. Phthalate esters are identified by their retention times. Confirmation is necessary and shown for target compounds by displaying the expected retention time on both columns. Method detection limits below 1 ppb are possible using this method.

15.12.2.4 Nitrosamines, Nitroaromatics, and Cyclic Ketones

Nitrosamines, which are known carcinogens, can be determined by USEPA SW846 Method 8070A (13). Only three nitrosamines, *N*-nitrosodimethylamine, *N*-nitrosodiphenylamine, and *N*-nitrosodi-*n*-propylamine, are listed in the method. Samples are extracted with methylene chloride using a USEPA technique. The extract is washed with dilute hydrochloric acid to remove coextracted amines. Two packed columns are recommended for the gas chromatographic separation, but capillary columns may be substituted. The packed columns are—1.8 m × 4 mm i.d. glass packed with Chromosorb W AW (80/100 mesh) coated with Carbowax 20M/2% KOH and 1.8-m × 4-mm-i.d. glass packed with Supelcoport (100/120 mesh) coated with 10% SP-2250. The thermal energy analyzer (TEA) is listed as the most sensitive and selective detector, but the NPD or the ELCD, operated in the reductive nitrogen mode, can be substituted.

USEPA SW846 Method 8091 allows for separation and detection of ppb concentrations of nitroaromatic and cycloketone compounds in soil and water samples (13). Thirty-six compounds are listed in the method. Water samples are extracted using liquid–liquid or continuous liquid extraction between pH 5 and 9. Soils are extracted by sonication or Soxhlet extraction. The extract can be cleaned up using Florisil SPE or GPC and changed over to hexane. The gas chromatographic conditions for the method are given in Table 15.14. The columns, as in the previous examples, are connected with a Y-shaped fused-silica connector and each interface into an ECD. Tentative identification of an analyte occurs when a peak falls in the retention time window. Confirmation is made if the analyte peak is also observed in the retention time window on the second column.

TABLE 15.14 Gas Chromatographic Conditions for USEPA Method 8091, Nitroaromatics, and Cyclic Ketones

Injector temperature	250°C
Injection volume	2 μL
Helium carrier flow	6 mL/min
Column 1: DB-5, 30-m × 0.53-mm × 1.5-μm film	
Column 2: DB-1701, 30-m × 0.53-mm × 1.0-μm film	
Oven temperature: 120°C for 1.0 min, ramp to 200°C at 3°C/min and hold for 1 min, ramp to 250°C at 8°C/min and hold for 4 min	
ECD temperature	320°C
ECD makeup (nitrogen)	20 mL/min

Source: Taken from Method 8091, Reference 13.

15.12.2.5 Phenols

USEPA SW846 Method 8041 offers a number of alternatives for the determination of phenols in water and soil samples (13). Nearly 40 phenolic compounds are listed in this method. Phenols are extracted from water at low pH (<2) with methylene chloride using liquid–liquid or continuous liquid extraction. Soils can be extracted using the sonication or Soxhlet extraction with 1–1 methylene chloride–acetone. After solvent evaporation and exchanging the solvent to 2-propanol, the phenols may be analyzed by FID using a single-column or dual-column approach; however, sensitivity may not be adequate for the underivatized phenols. The phenols can be derivatized with diazomethane to form the anisole or methyl ester of the phenol and determined by FID. The best sensitivity and selectivity can be obtained, however, by derivatizing the sample extracts with pentafluorobenzylbromide (PFBBr) and detecting the derivatized phenols using ECD. Three phenols—2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, and Dinoseb—are not derivatized by PFBBr. A silica gel cleanup is used after the derivatization. Dual megabore columns (DB-5 and DB-1701 (see Tables 15.13 and 15.14) are recommended for the separation.

15.12.3 Petroleum Fingerprinting of Contaminated Soils and Water Using GCFID

Petroleum distillates heavier than gasoline, such as diesel fuel, fuel oils, and mineral spirits, can leak or spill into the environment. It is often necessary to identify and quantify these petroleum distillates, referred to as total petroleum hydrocarbon (TPH) in soil and water for site assessment or remedial action. Decisions on and the amount of cleanup necessary for the contaminated site will be risk-based corrective action (RBCA) defined by the National TPH Criteria Working Group of the USEPA. Since these distillates are multi-componented, they appear as a recognizable pattern or range of peaks in chromatograms generated from a GCFID. Sometimes this pattern is called a "fingerprint." The methods for determining these heavier petroleum distillates are similar to those used for determining GRO (see Section 15.11.3). These compounds are too heavy and nonvolatile for dynamic headspace extraction and, therefore, a liquid extraction is necessary for waters and for soils.

In the application that is presented below, soil (20 g) from around a leaking tank was dried with sodium sulfate and extracted in a vial by shaking it with 20 mL of pentane. Two surrogate standards were added: *o*-terphenyl for the aromatic fraction and chlorooctadecane for the aliphatic fraction. A portion of the extract was injected directly into a GCFID. The extract was fractionated into the aliphatic and aromatic fraction using a 3-g silicagel column. One milliliter of the extract was placed on the head of the silicagel column after it had been washed with 40 mL of methylene chloride and 40 mL of pentane. The aliphatic fraction was first eluted using approximately 10 mL of pentane and collected. The aromatic was eluted with approximately 10 mL of 2–3 methylene chloride–pentane (v/v) and collected. Both extracts were evaporated to 1.0 mL and injected into the GCFID. The operating conditions for the GCFID are given in Table 15.15.

TABLE 15.15 Gas Chromatographic Conditions for Determination of Petroleum Hydrocarbons in Soil

Injector temperature	300°C
Injection technique	Direct
Injection size	1 μL
Column	SPB-5, 30-m × 0.32-mm × 0.25-μm film
Hydrogen carrier gas	10 mL/min
Oven temperature	35°C for 2 min, ramp at 10°C/min to 300°C, and hold for 6.5 min
FID temperature	320°C

Chromatograms generated using this method for a standard and the three fractions from the soil sample are shown in Figure 15.18. The standard (top figure) shows aliphatic hydrocarbons over the range from C8 to C40 and selected aromatic hydrocarbons and PAHs. Accurate quantification from standards is possible of unknown hydrocarbons in segmented ranges based on carbon number, because the response of the FID is essentially the same for all hydrocarbons and based primarily on the effective carbon number (93). This standard is integrated over certain carbon ranges to obtain the FID response that is consistent for hydrocarbons found in that range. For example, the diesel range organics are taken from C10 to C20. The total peak area from C10 to C20 would then be compared against the response factor of the standard in that range to quantify GRO. In the examples shown above in Figure 15.18, no distinctive hydrocarbon pattern is obvious. The total, aromatic, and aliphatic hydrocarbon fractions were all integrated for a number of carbon ranges, marked by *n*-alkanes in the chromatogram, from C8 to C35. The areas from the surrogate standards were subtracted from the hydrocarbon areas prior to quantification. Quantification was performed for all three fractions over seven carbon ranges, including the total range from C8 to C35. This quantitation for the three sample fractions is shown in Table 15.16.

In this example the aliphatic fraction contributed more to the total hydrocarbon concentration than did the aromatic fraction. When remedial action is performed based on RBCA on a contaminated area, the aromatic fraction, which is considered to pose the greatest threat, because of mobility and toxicity, will weigh heaviest in determining the extent of cleanup that is necessary. Usually hydrocarbons beyond C35 are ignored in RBCA, because their lack of volatility and mobility in the environment makes them a lower risk to human and animal health. In this example the carbon ranges overlap because of the different reporting requirements of the different state agencies.

15.12.4 Methods for Determining Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans

Dioxin is the widely used term that is used by the public to describe the polychlorinated dibenzo-*p*-furans (PCDFs) and polychlorinated dibenzo-*p*-dioxins

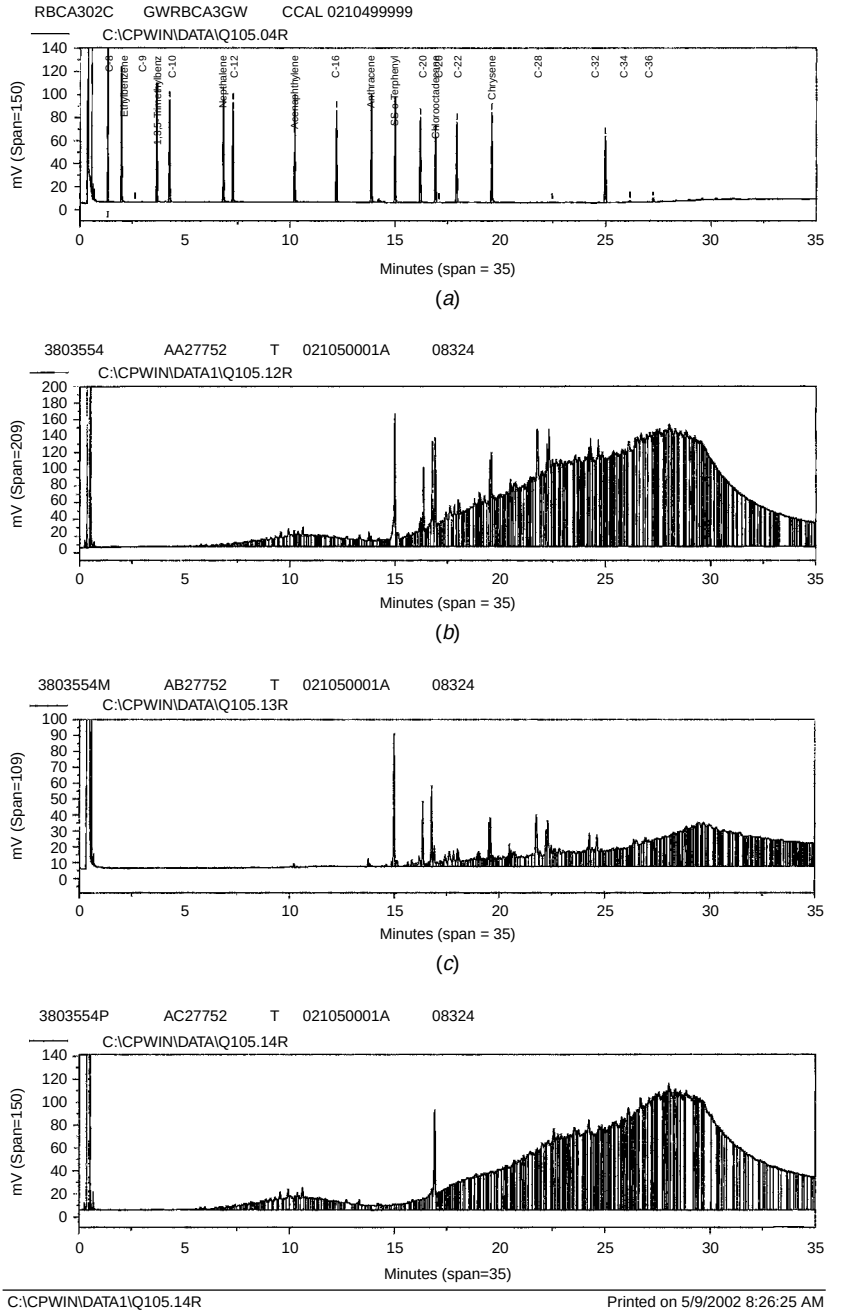


FIGURE 15.18 Chromatograms of soil contaminated with fuel from LUST using GCFID; method conditions defined in Table 15.15: (a) TPH standard; (b) total petroleum hydrocarbon; (c) aromatic fraction; (d) aliphatic fraction (courtesy of Lancaster Laboratories).

TABLE 15.16 Quantification of Total Petroleum Hydrocarbons, Aromatic Hydrocarbons, and Aliphatic Carbons from C8 to C35 Based on *N*-Alkane Markers for RBCA

	Total Petroleum Hydrocarbon (ppm)	Aromatic Fraction (ppm)	Aliphatic Fraction (ppm)
>C8–C10	1.4	0.3	0.3
>C10–C12	9.0	0.2	7.0
>C12–C16	120	4.7	96
>C16–C21	210	37	84
>C21–C28	800	97	450
>C21–C35	2000	230	1200
Total > C8–C35	2400	270	1400

(PCDDs). Two USEPA SW846 Methods, 8280A and 8290, outline procedures for determining the congeners of the PCDFs and the PCDDs in environmental samples (13). Although 210 congeners are possible, the 17 congeners that are substituted in the 2, 3, 7, and 8 positions are considered most toxic and of analytical interest. Both methods use a high-resolution gas chromatographic separation with 60-m, narrow-bore capillary columns. Method 8280 uses low-resolution mass spectrometry and selective-ion monitoring. Method 8290 uses high-resolution mass spectrometry and SIM. The high-resolution mass spectrometer must have a resolving power of at least 10,000 for this method. Both methods provide procedures for the detection and quantification for homologs containing four (tetra-) through eight (octa-) chlorine atoms. The sample preparations are similar, but are extremely tedious and detailed in both methods. Soil, flyash, and chemical wastes are extracted with toluene using a Soxhlet apparatus containing a Dean–Stark water trap. Water samples are extracted using methylene chloride. Method 8390 also describes procedures for tissue samples and paper pulp. Internal standards using C13 analogs of PCDDs and PCDFs are added to samples prior to the extraction. Sample extracts are concentrated to approximately 10 mL and washed with the following: concentrated sulfuric acid, 5% sodium chloride solution, 20% potassium hydroxide solution, and 5% sodium chloride solution. The organic extract is switched to hexane and evaporated to 1.0 mL and passed through a silicagel column and then an alumina column. Hexane and 20% methylene chloride/hexane are used to elute the columns. The extract is evaporated to 2–3 mL and passed through an activated carbon/Celite column. Extracts are taken to a final volume of 100 μL in tridecane or nonane. The primary column for both methods is a DB5, 60-m × 0.25-mm × 0.25- μm film. The identification of the target compounds is based on their ordered elution and comparison to standard solutions. Four SIM descriptors are used in Method 8380 and five SIM descriptors are used in Method 8390 to monitor the tetra- through octa-homologues of the PCDFs and the PCDDs. Resolution of the all of the 2,3,7,8-specific isomers can not usually be achieved on a single column. If samples are found to have a sufficient toxicity equivalent concentration (TEC) (94), then

reanalysis using a 60-m SP-2331, SP-2330, or DB225 column may be required. A five-level internal standard calibration, ranging from 0.1 to 2.0 ng/μL, is used for the low-resolution MS method. Quantitation limits for water samples range from 10 L to 50 ng/L (ppt) and for soils and flyash range from 1.0 to 5.0 μg/kg (ppb) in the low-resolution method. Reporting limits in the low part per trillion are realized for the high-resolution mass spectrometry method.

15.13 DETERMINATION OF PESTICIDES AND POLYCHLORINATED BIPHENYLS

15.13.1 Organochlorine Pesticides and PCBs Using the Electron-Capture Detector

The electron-capture detector (ECD) is very sensitive and selective to chlorinated compounds. Because of this it has been used extensively in the analysis of environmental samples for the determination of chlorinated pesticides and PCBs. Method 608 was one of the first USEPA methods for organochlorine pesticides and PCBs and used packed columns and the ECD (12). The method still references packed columns, but allows for the use of open tubular capillary columns. In reality nearly all pesticide separations are now performed on capillary columns because of improved resolution, increased sensitivity, and faster analysis times. The USEPA SW846 method that is used heavily by environmental laboratories is Method 8081A (13). Method 8082 is used for PCBs, although the instrument setups and sample preparation procedures are nearly identical for the two methods (13). Water samples (1 L) are extracted with methylene chloride using the liquid–liquid shakeout or the continuous liquid extraction. Soil samples (30 g) or solids are usually extracted by sonication or Soxhlet using 1–1 methylene chloride–acetone. SPE cleanups using Florisil or alumina are often necessary. Gel permeation chromatography or sulfur cleanup is often necessary with the ECD. Extracts are switched to hexane to be compatible with the ECD and evaporated to a final volume of 10 mL.

Dual-column confirmation or GCMS confirmation is required for most USEPA pesticide methods. GCMS is seldom sensitive enough to detect the low concentrations of organochlorine pesticides and PCBs detected by the ECD. Dual-column confirmation means that a peak can be identified as a target pesticide if it is present in the expected retention time window on two columns with different stationary phases. Because it is more efficient to do the analysis and confirmation simultaneous, many gas chromatographs are configured with dual capillary columns that are connected onto a guard column with a fused-silica Y connector. The carrier gas flow from the injection port is spilt between two columns of equal length and diameter, but having different phases. Each column is then interfaced to an ECD. Approximately 30 organochlorine pesticides are listed in Method 8081A, a number of the compounds are breakdown products or metabolites of the pesticides. For example, 4,4′-DDE and 4,4″-DDD are metabolites of 4,4′-DDT and endrin aldehyde (EA) and endrin ketone (EK) are metabolites of endrin. Example gas chromatographic conditions for determining the organochlorine pesticides by dual-column and ECD are shown in Table 15.17.

TABLE 15.17 Gas Chromatographic Conditions for USEPA Method 8081A, Organochlorine Pesticides by Dual-Column GCECD

Injector temperature	275°C
Injection volume	1 μL
Injection technique	Direct
Hydrogen carrier	6–10 mL/min per column
Oven temperature: 140°C and ramp at 15°C/min to 300°C, hold for 6 min	
Column 1: CLP1, 30-m × 0.32-mm × 0.50-μm film	
Column 2: CLP2, 30-m × 0.32-mm × 0.25-μm film	
ECD temperature	300°C
ECD makeup (nitrogen)	30 mL/min

Example chromatograms of the common organochlorine pesticides separated by these conditions are shown in Figure 15.19. The chromatograms are generated from a continuous calibration standard in hexane with each pesticide in the range of 10–20 ng/mL. The surrogate standards, tetrachloro-*meta*-xylene (TCX) and decachlorobiphenyl (DCB), are present at approximately 40 ppb.

The Rtx-CLPesticides (CLP1) and Rtx-CLPesticides2 (CLP2) are columns with proprietary phases developed by a commercial vendor to specifically separate the organochlorine pesticides. These phases allow the separation of the complete list of organochlorine pesticides in a single injection. Other phases that are commonly used are DB-608, DB-5, and DB-1701. In the application above, an external standard calibration is performed on the basis of peak height because of the complicated nature of the chromatograms. An internal standard calibration and peak area may be used. A five-level calibration over a 20-fold calibration range, where the lowest standards are 1 or 2 ng/mL, is used. Sub-ppb detection limits are achieved for water and soil.

An important method performance test is often required for the organochlorine pesticides. DDT and endrin will degrade in the injection port and at the head of the column. Accumulation of nonvolatile residue from sample extracts and hot metal surfaces can cause this breakdown. Prior to analyzing samples a mixture of endrin and DDT are injected and the percent breakdown for DDT and endrin are calculated as

$$\%\text{breakdown DDT} = \frac{\text{peak Areas (DDE + DDD)}}{\text{peak areas(DDT + DDE + DDD)}} \times 100\% \quad (15.11)$$

$$\%\text{breakdown endrin} = \frac{\text{peak areas (EA + EK)}}{\text{peak areas(endrin + EA + EK)}} \times 100\% \quad (15.12)$$

Method 8081A requires these breakdowns to be less than 20%.

The PCBs are extracted and cleaned up in the same manner as the organochlorine pesticides because of their similar chemical properties, and often the same extracts are used for analysis. The instrument setups are identical in many cases. The PCB Aroclors are multicomponent mixtures containing chlorinated congeners. The USEPA SW846 Method 8082 lists seven Aroclor mixtures (1016,

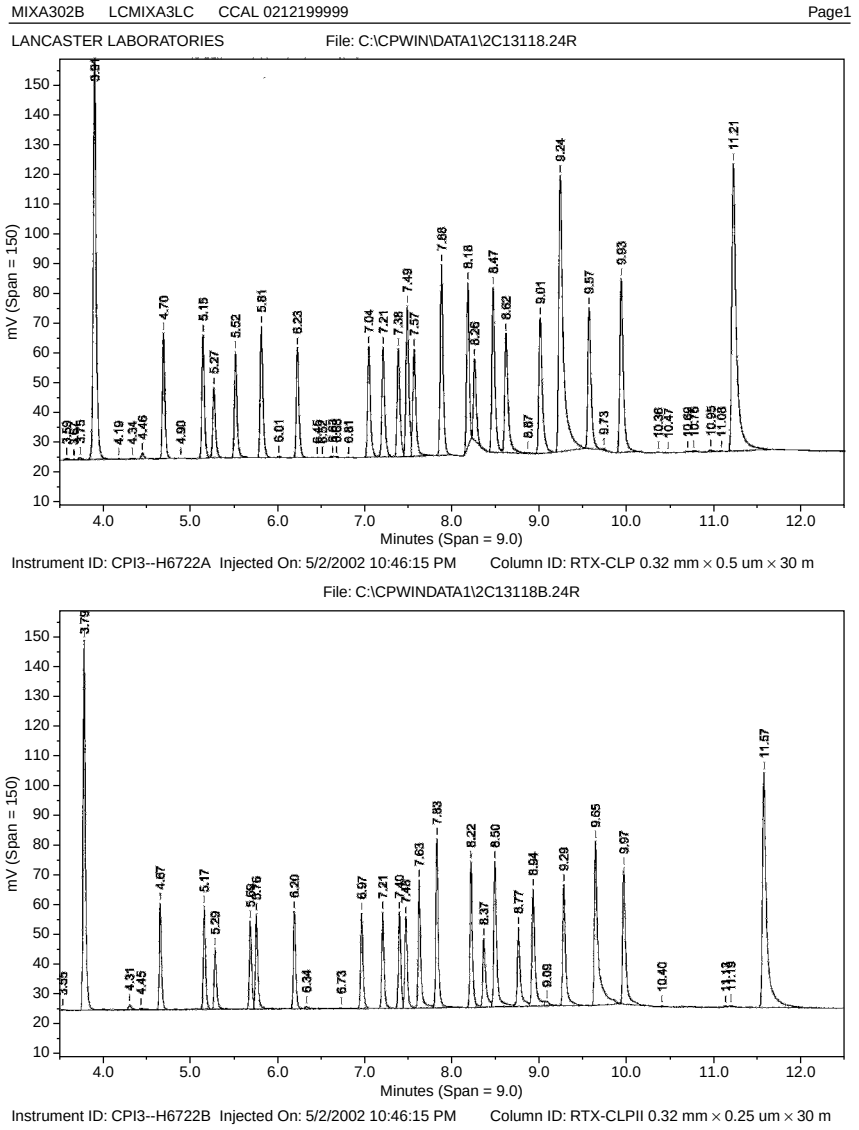

MIXA302B LCMIXA3LC CCAL 0212199999 Page1
LANCASTER LABORATORIES File: C:\CPWIN\DATA1\2C13118.24R
mV (Span = 150)
Minutes (Span = 9.0)
Instrument ID: CPI3--H6722A Injected On: 5/2/2002 10:46:15 PM Column ID: RTX-CLP 0.32 mm × 0.5 um × 30 m
File: C:\CPWINDATA1\2C13118B.24R
mV (Span = 150)
Minutes (Span = 9.0)
Instrument ID: CPI3--H6722B Injected On: 5/2/2002 10:46:15 PM Column ID: RTX-CLPII 0.32 mm × 0.25 um × 30 m

FIGURE 15.19 Chromatograms (GCECD) of common organochlorine pesticides by USEPA Method 8081A, continuous calibration standard, pesticides at 10–20 ppb; instrument conditions given in Table 15.17 (courtesy of Lancaster Laboratories, Pesticide Group).

RT CLP1 (min)	RT CLP2 (min)	Compound Name	Conc. ng/mL
3.91	3.79	TCX	40
4.70	5.17	α-BHC	10
5.15	5.17	γ-BHC	10
5.27	5.29	β-BHC	10
5.52	5.70	δ-BHC	10
5.82	5.76	heptachlor	10
6.23	6.20	aldrin	10
7.05	6.97	hept. epoxide	10
7.21	7.21	γ-chlordane	10
7.38	7.40	α-chlordane	10
7.57	7.48	endosulfan I	10
7.49	7.63	4,4'-DDE	20
7.88	7.83	dieldrin	20
8.18	8.23	endrin	20
8.26	8.37	4,4'-DDD	20
8.47	8.50	endosulfan II	20
8.62	8.77	4,4'DDT	20
9.01	8.94	endrin aldehyde	20
9.24	9.65	methoxychlor	100
9.57	9.29	endosulfan sulf.	20
9.93	9.97	endrin ketone	20
11.21	11.57	DCB	40

FIGURE 15.19 (*Continued*)

1221, 1232,1242, 1248, 1254, and 1260) and 19 PCB congeners that can be determined by the method (13). When the Aroclor mixtures are used for calibration standards, an individual standard is made for each Aroclor mixture, because congeners can overlap in the mixtures. Sometimes Aroclor 1016 and Aroclor 1260, however, are combined. Typically three to five peaks from individual congeners are chosen for quantification. The peak areas or heights are summed to obtain an overall response factor for the Aroclor. Pattern recognition and retention times are used to identify the Aroclor mixtures in samples. As long as no interfering peaks are present, the same set of peaks as chosen for the Aroclor standard, are summed and used for quantification of samples. Pattern recognition can be difficult in complex chromatograms and when the Aroclor mixture has been weathered in the environment. Example chromatograms of an Aroclor 1260 standard (combined with Aroclor 1015) and a soil sample Aroclor 1260 are shown in Figure 15.20. The instrumental conditions and columns are identical to those listed above in Table 15.17 for the organochlorine pesticides except that oven temperature of the gas chromatograph was started at 160°C and ramped at 150°C/min to 300, and held for 4 min. Only the chromatograms from the CLP1 column are displayed.

The 1260 pattern is readily apparent on the soil, which has major peaks at 4.84, 5.62, 5.85, 6.18, 6.52, 6.66, 6.96, 7.34, 7.69, 7.73, 7.79, 8.46, and 9.04 min, which are also observed in the Aroclor 1260 standard. All of these peaks could have been used for quantification; however, only five were chosen. The amount

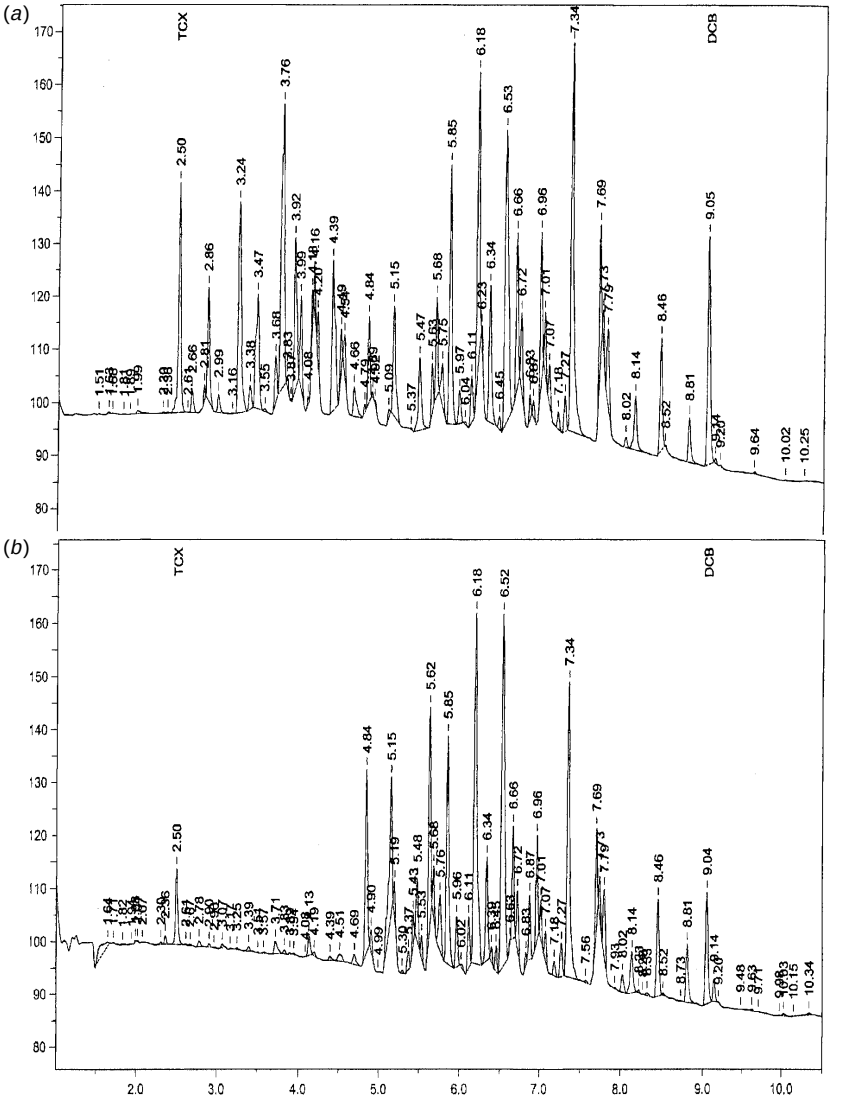


FIGURE 15.20 PCB chromatograms by GCECD using pattern recognition: (a) Aroclor 1260 standard at 0.5 ng/μL; (b) Aroclor 1260 contaminated soil; see Table 15.17 for instrumental conditions (courtesy of Lancaster Laboratories, Pesticide Group).

of Aroclor 1260 found in this particular soil was 1.2 ppm. TCX and DCB are added as surrogate standards prior to extraction for the PCBs. Method detection limits for the Aroclor mixtures range from approximately 0.05 to 1.0 μg/L in water and 60 to 70 μg/kg in soil.

Other USEPA methods for organochlorine pesticides include the Contract Laboratory method under CERCLA or Superfund (15,16), Method 508 (95),

and Method 508.1 (96) for drinking water. Although Method 508.1 uses the solid-phase extraction for water samples, all these methods use GCECD and are very similar.

15.13.2 Gas Chromatographic Methods to Determine Organophosphorus Pesticides Using the Nitrogen Phosphorus Detector and the Flame Photometric Detector

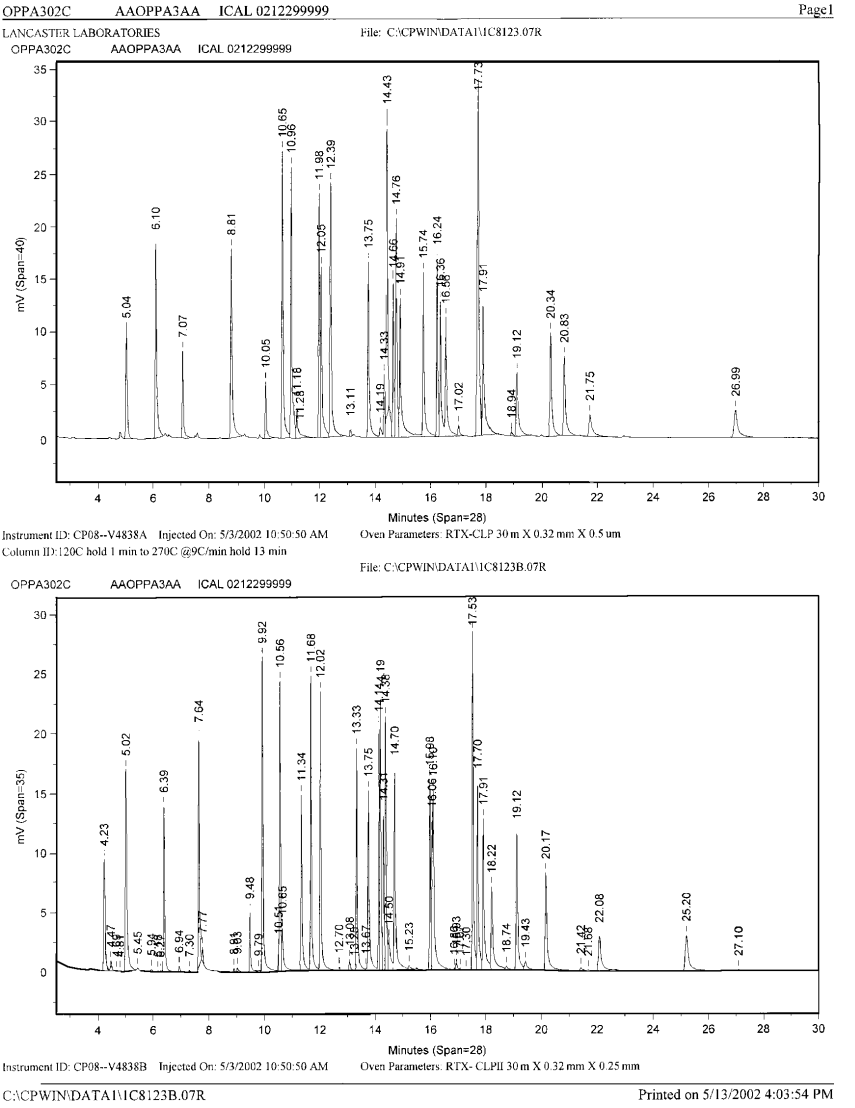
USEPA Method 8141A describes the capillary gas chromatographic method for determining the organophosphorus pesticide using either the nitrogen–phosphorus detector (NPD) or the flame photometric detector (FPD) (13). Both detectors are very selective to organic compounds containing phosphorus. The FPD is also selective to compounds with sulfur, while the NPD is also selective to nitrogen-containing compounds. When the NPD is used, the triazine herbicides are often included with the organophosphorus pesticides on the target list. Approximately 50 organophosphorus pesticides are included on the target list for 8141A. Samples are prepared in the same many as the organochlorine pesticides (see text above). The sonication extraction of soils is not advised for the organophosphorus pesticides, however, because of the potential for destroying these compounds during ultrasonication (97). The organophosphorus pesticides are much more fragile than the organochlorine pesticides. They undergo hydrolysis in acidic and basic conditions, and many photodecompose. A Florisil cleanup or GPC can be used for the organophosphorus pesticides. Extracts are concentrated to a final volume of 10 mL in hexane to be compatible with the NPD. Example chromatograms for the organophosphorus pesticides using a dual-column GCNPD method are shown in Figure 15.21. The same CLP1 and CLP2 columns as listed in Table 15.17 were used for the separation of this midlevel calibration standard (concentration $\sim$ 500 ng/mL for most analytes).

Other stationary phases such as the SPD-608 and the DB-210 are recommended in Method 8141A. The cyanopropyl phase (DB-1701), however, can cause elevated baselines when used with the NPD, because of the nitrogen it contains. A normal calibration range for the organophosphorus pesticide standards is 200–1000 ng/mL. Method detection limits are in the low parts per billion for water and soil samples. USEPA Method 507 uses capillary GCNPD to determine the organophosphorus pesticides in drinking water (98).

15.13.3 High-Resolution Separation of PCB Congeners with Electron-Capture Detection

Approximately 150 of the possible 209 PCB congeners have been found in the environment. Currently, because all 209 PCB congeners have been synthesized and are available as standards, and because of technical advances in capillary gas chromatography, the determination of the individual PCB congeners in environmental samples has been made possible and has several advantages over determination of the technical-grade Aroclors. Congener-specific analysis

allows for the detection of the more toxic PCB congeners. Congeners, which lack chlorine substitution in the *ortho* position, can align in a planar configuration (e.g., PCB 77) and show a particularly high toxicity much like the polychlorinated dioxins and furans. The coplanar PCBs are also suspected of being endocrine disrupters (99). These planar congeners are not easily detected


OPPA302C AAOPPA3AA ICAL 0212299999 Page1
LANCASTER LABORATORIES File: C:\CPWIN\DATA1\1C8123.07R
OPPA302C AAOPPA3AA ICAL 0212299999
mV (Span=40)
Minutes (Span=28)
Instrument ID: CP08--V4838A Injected On: 5/3/2002 10:50:50 AM Oven Parameters: RTX-CLP 30 m X 0.32 mm X 0.5 um
Column ID:120C hold 1 min to 270C @9C/min hold 13 min
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OPPA302C AAOPPA3AA ICAL 0212299999
mV (Span=35)
Minutes (Span=28)
Instrument ID: CP08--V4838B Injected On: 5/3/2002 10:50:50 AM Oven Parameters: RTX- CLPII 30 m X 0.32 mm X 0.25 mm
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FIGURE 15.21 Organophosphorus pesticides using dual columns and the NPD. Conditions: injector 275°C; carrier gas, hydrogen at approximately 4–5 mL/min through each column, CLP1 and CLP2; see Table 15.17; oven, 120°C for 1.0 min, and ramp to 270°C at 9°C/min, and hold for 13 min. (Courtesy of Lancaster Laboratories, Pesticide Group).

RT CLP1 (min)	RTCLP2 (min)	Compound Name	Conc. ng/mL
5.04	4.23	TMX	5000
6.09	5.02	dichlorvos	500
7.07	6.39	1-bromo-2-nitrobenzene	10000
8.81	9.48	mevinphos	500
10.05	9.48	demeton-O	100
10.65	9.92	Ethoprop	500
10.97	10.56	phorate	500
11.18	10.65	naled	500
12.05	11.34	demeton-S	500
11.98	11.68	diazinon	500
12.39	12.02	disulfoton	500
13.79	13.33	ronnel	500
14.66	13.75	methyl parathion	500
------	14.14	dursban	500
14.77	14.19	tricholate	500
14.91	14.31	malathion	500
14.33	-----	merphos oxone	100
14.43	14.38	fenthion	500
15.75	14.71	ethyl parathion	500
16.24	15.98	tokuthion	500
16.56	16.06	stirophos	500
16.36	16.10	merphos	500
------	15.53	ethion	500
17.73	17.71	bolstar	500
17.91	17.91	trithion	500
19.12	18.22	fensulfothion	500
20.34	19.12	famphur	500
20.83	20.17	EPN	500
21.75	22.08	guithion	500
26.99	25.20	coumaphos	500

FIGURE 15.21 (*Continued*)

by classical pattern recognition methods because of their low levels compared to the bulk PCB and their coelution with one or more interfering congeners (100). The congener-specific analysis also allows for better tracking of the PCBs in the environment. PCB congeners migrate in the environment and are accumulated into the food chain at different rates. For example, Aroclor 1260, released into the environment in the 1970s, may not be detected by classical pattern recognition methods in soil or animal tissue because of weathering and distortion of the Aroclor pattern (101). The congener specific analysis has also been shown to be more accurate and less biased than the traditional Aroclor method in quantifying PCBs. Significant error is introduced into the traditional multicomponent PCB

analysis because of variable ECD response to isomeric PCBs and the presence of more than one congener in a single peak (102).

Separation of all 209 congeners is an extremely difficult and tedious task. This has not been done on a single capillary column. Usually dual, narrow-bore (0.25 mm or 0.32 mm i.d.) capillary columns 30 or 60 m in length, are used for the high-resolution separation of the congeners. Two columns with a nonpolar and a moderately polar phase, connected in parallel with two ECDs, is a typical arrangement. Frame and others present a collaborative study of the 209 PCB congeners and six Aroclors on 20 different high-resolution gas chromatographic columns using GCECD and GCMS (103). Usually only those congeners with environmental significance, such as the planar PCB congeners mentioned, are monitored. In Figure 15.22 the congeners present in Aroclor 1254 are separated using a very nonpolar capillary column: SPB-Octyl (50% n-octyl, 50% methylpolysiloxane), 30-m × 0.25-mm × 0.25-μm film. Peaks in Figure 15.22 are labeled by their congener number; see Reference 21.

15.13.4 Alternate Methods for the Determination of Pesticides and PCBs

One of the major impediments to the use of GCMS for the determination of pesticides and PCBs has been that the sensitivity has not been comparable to that

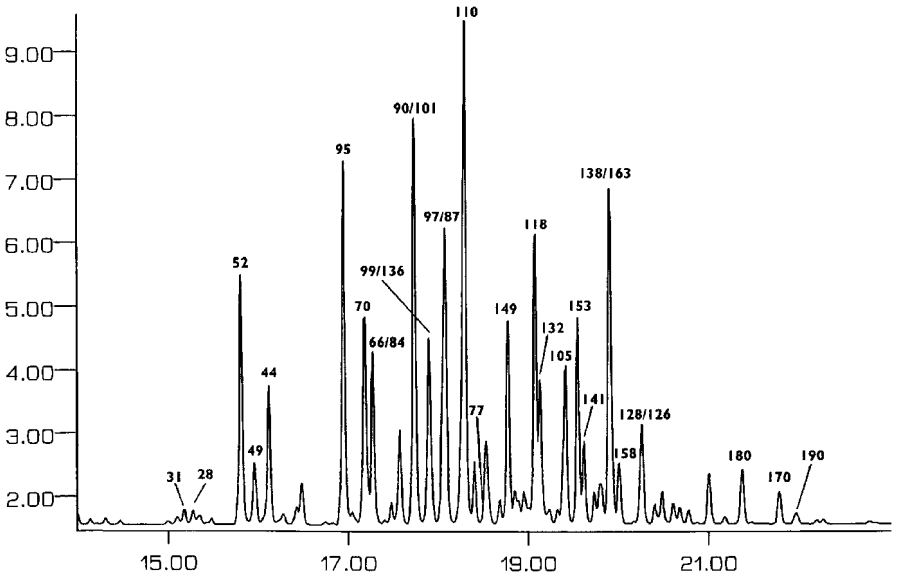


FIGURE 15.22 PCB congeners in Aroclor 1254 by GCECD. Conditions: injection, 1 μL splitless (280°C, split at 30 s); carrier gas, helium 35 cm/s; oven, 150°C for 1 min, and ramp to 280°C at 4°C/min, and hold for 20 min; ECD temperature 300°C. (Courtesy of Supelco, Reference 104.).

of the ECD or NPD possible. USEPA Method 680 determines 22 organochlorine pesticides and the PCB congeners using GCMS (105). The method describes using the GCMS in both the scan mode and in the selective-ion monitoring (SIM) mode. Increased sensitivity can be achieved using the mass spectrometer in the SIM mode. Method detection limits are significantly higher, however, than can be achieved using the ECD. The new generation of mass spectrometers has shown increased sensitivity and is beginning to compete against the ECD. Another approach that has shown promise has been to use large volume injections in conjunction with the GCMS. In this approach a large volume of extract is injected and the solvent is vented using a specially designed injection port prior to the separation on a capillary column. Injection volumes of 50–100 μL are typical, but volumes of 1.0–2.0 mL have been achieved. The increased loading of the analyte onto the column has lowered detection limits proportionally.

15.14 GAS CHROMATOGRAPHIC METHODS USING DERIVATIZATION TO DETERMINE NONVOLATILE COMPOUNDS AND CHLORINATED ACID HERBICIDES

The number of organic compounds that are amenable to gas chromatographic separation is limited by volatility and polarity. Organic compounds that can not partition into the gas mobile phase will not pass through the column and reach the detector. Compounds that are extremely polar will bind to active sites in the chromatographic system and will tail excessively, preventing accurate quantification and even detection. Two classes of compounds, the chlorophenoxy acid herbicides and the haloacetic acids, which are byproducts from the disinfection of water, can be methylated and separated by gas chromatography. Normally these compounds are too ionic for gas chromatography. Methods to determine these compounds in environmental samples are discussed in the following sections.

15.14.1 Chlorinated Acid Herbicides

USEPA Method 8151A lists 19 compounds on the target list. Because these compounds are protonated in water at low pH and exist in the anionic state at higher pH, water samples are cleaned up using a base partitioning at pH 12 or greater. After adding the surrogate standard [2,4-dichlorophenylacetic acid (DCAA)] and 250 g of sodium chloride to one liter of water, the pH is adjusted to pH 12 with sodium hydroxide. The sample is washed with three 60-mL portions of methylene chloride, which are discarded. After adjustment to pH 2 or less with sulfuric acid, the sample is extracted with a 120-mL portion of ethyl ether and two 60-mL portions. The ethyl ether is dried by passing it through acidified sodium sulfate. The extract is evaporated down to a final volume of 4 mL containing ethyl ether, 0.5 mL methanol, and 1.0 mL of isooctane. Bubbling diazomethane gas through the extract methylates the herbicides. After adding silicic acid to destroy any remaining diazomethane, the extract is taken to a final volume of 10 mL. Soil samples are pH adjusted to less than 2 and mixed with acidified

sodium sulfate. Soils can be extracted ultrasonically or with a shaker extraction. In the ultra sonic extraction the soil is extracted with three 60-mL portions, which can be cleaned up with a base partitioning. If the base partitioning is not needed, the soil extract is dried with acidified sodium sulfate and evaporated, and the solvent is exchanged for methylation in the same manner as the waters (13).

The methylated herbicides are separated and detected using dual columns and two electron-capture detectors. This setup is identical to the configuration described above for the organochlorine pesticides. Example chromatograms of a laboratory control sample (LCS) are shown in Figure 15.23. The LCS was prepared by spiking laboratory reagent water with a methanolic mixture of the herbicides and carrying it through the extraction and methylation. Although the concentration of the herbicides in the LCS is compound-specific and related to detector response, they ranged from 0.2 to 250 μg/L.

The internal standard was 4,4′-dibronooctafluorobiphenyl (DBOFB). The calibration was based on five levels of standards, and peak height was used for quantification. USEPA Methods 515.1 and 515.2 can also be used to determine the herbicides by GCECD (106,107). Both methods are very similar to USEPA SW846 Method 8151A and rely on methylation of the acid herbicides, but Method 515.1 uses the classical liquid–liquid extraction but in Method 515.2 water samples are extracted using SPE.

15.14.2 Haloacetic Acids

USEPA Method 551.2 addresses the determination of neutral disinfection byproducts and includes trihalomethanes, haloacetonitriles, chlorinated solvents, and chlorinated pesticides on its target compound list. A microextraction technique using MTBE or pentane (3 or 5 mL, respectively) is used for water samples (50 mL). Analysis is performed using capillary columns and GCECD (108). Method 552.2, however, is for the determination of the haloacetic acids. The following compounds are on the target list of analytes: bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), chlorodibromoacetic acid (CDBAA), Dalapon, Dibromoacetic acid (DBAA), Dichloroacetic acid (DCAA), monobromoacetic acid (MBAA), monochloroacetic acid (MCAA), tribromoacetic acid (TBAA), and trichloroacetic acid (TCAA). A 40-mL sample is adjusted to a pH less than 0.5 and extracted with 4 mL of MTBE. The haloacetic acids in the extract are converted to their methyl esters using acidic methanol. Analysis is by capillary GC and ECD (109).

15.15 GAS CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF ORGANOMETALLIC COMPOUNDS

Many organometallic species exist in the environment and range from being nontoxic to extremely toxic. Speciation and detection of these compounds has been

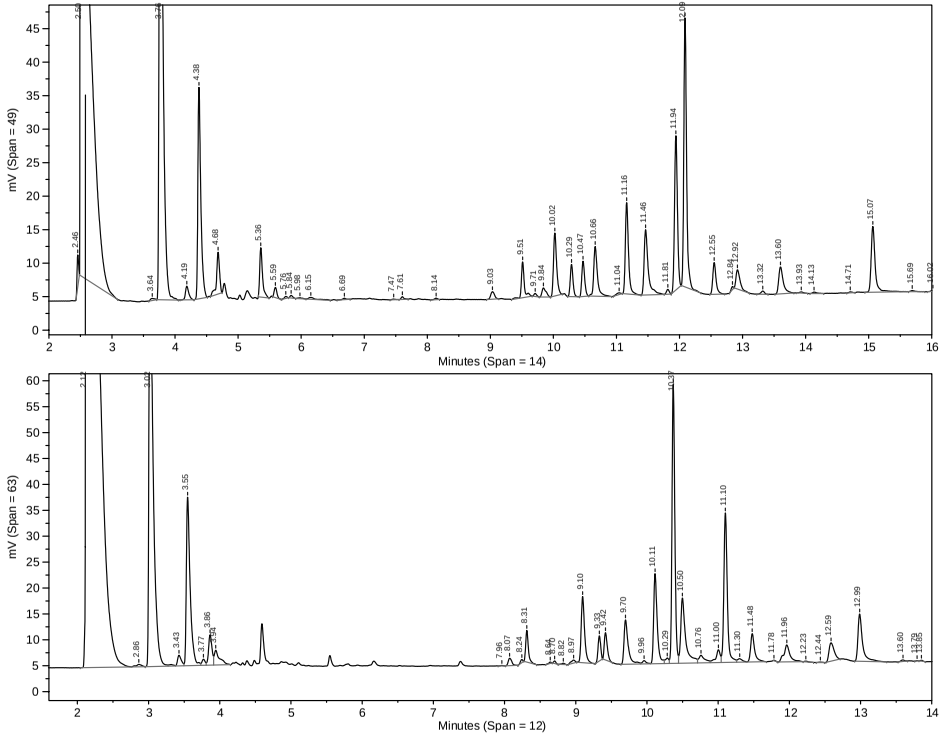


FIGURE 15.23 Separation of chlorinated acid herbicides in a water (LCS) using dual-column GCECD. Conditions: injection, 1 μL direct (280°C); top column (1), CLP1 30-m × 0.32-mm × 0.5- μm film; bottom column (2) CLP2, 30-m × 0.32-mm × 0.25- μm film; carrier gas, hydrogen at approximately 5 mL/min through each column; oven, 85°C for 2 min, ramp at 30°C/min to 170°C, ramp at 10°C/min to 270°C, and hold for 14 min.; ECD temperature 300°C. (Courtesy of Lancaster Laboratories' Pesticide Group.).

Compound	RT CLP1 (min)	RT CLP2 (min)
Dalapon	4.38	3.55
DCAA (SS)	10.02	9.10
Dicamba	10.29	9.33
MCPP	10.47	9.42
MCPA	10.66	9.70
2,4-DP	11.16	10.11
DBOFB(IS)	12.09	10.37
2,4-D	11.46	10.50
PCP	11.94	11.01
2,4,5-TP	12.55	11.48
2,4,5-T	12.92	11.48
2,4-DB	13.60	12.59
Dinoseb	15.07	12.99

FIGURE 15.23 (*Continued*)

of increased concern since the early 1990s. Determining the species is important in environmental impact assessment because it affects bioavailability and mobility in the environment (110). Collection and analysis of the organometallic compounds must occur in such as way as to not change the state or ligands on the metal. Water samples are often extracted using liquid–liquid extraction or SPE (111). Solid samples are often extracted with liquids using the Soxhlet extraction. SFE also has been used successfully. Derivatization using a Grignard reagent or sodium tetraethylborate (112) may be necessary to create a gaseous species. A Grignard reagent has the form R-Mg-X, where X is a halogen and R is an organic moiety such as an alkyl. For example, C_2H_5MgBr can be used to derivatize dimethyl lead and dimethylethyl lead, which are monovalent cations found in the environment, to form one volatile species, dimethyldiethyl lead. Tetraethylborate can be used to ethylate ionic methyl mercury (CH_3Hg^+) and form a volatile species.

When gas chromatography is used to separate organometallic compounds, a number of detectors can be used. Common detectors such as the FID, FPD, ECD, and MS have been employed. The most sensitive and selective detector for organometallic species is the atomic emission detector (113). The effluent from the GC enters a small chamber, and a microwave radiation is used to generate plasma. The intensity of the atomic emission radiation from the metal is monitored at a specific wavelength. Sometimes gas chromatography is interfaced with other instrumentation, such as atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectrometry (ICPMS), to detect the metal species.

A few applications taken from the literature are presented in this section. Pereiro and others used a focused microwave field to hydrolyze, derivative, and extract mono-, di-, and tributyltin, and triphenyltin from biological samples. Sodium tetraethylborate was used to form the ethyl derivatives of these species, which partitioned into the supernatant organic phase. The extract was injected onto a capillary chromatographic column for separation and detection using the FPD and AED (114). Vazquez and others also used microwave-assisted extraction

to determine methylmercury in marine sediments. Capillary GCECD was used a obtain detection limit of 8 ng/g (115). Gorecki and Pawliszyn used SPME and GCFID and GCMS to determine tetraethyl lead (TEL) and inorganic lead in water. The TEL was determined by using SPME to sample the headspace over water samples. Inorganic lead was first derivatized with sodium tetraethylborate to form TEL and determined in the same manner. The detection limit for TEL was found to be 5 ppt when using GCMS (116). Gomez-Ariza and others speciated the volatile forms of selenium and inorganic selenium in sediments using GCMS (117). Volatile species of selenium were desorbed from sediments using a dynamic headspace method with activated carbon as the trap (117). Donais et al. developed, validated, and applied a method to quantify methyl mercury in biological marine materials using GCAED. A liquid–solid extraction with GPC was used on complex marine samples. Derivatization was found to be unnecessary to obtain stable chromatographic separations and quantification at the ng/g level in the marine samples (118).

15.16 ANALYSIS OF AIRBORNE POLLUTANTS

The gas chromatographic methods that have been discussed thus far are nearly all applicable to the analysis of airborne pollutants. The target analytes in the analysis of air are often the same as those pollutants that are found in water and soil. The major difference in the analysis of air samples from that of water and soil samples is the sampling process and introduction of the analytes into the gas chromatograph. Air is a mixture of gases containing approximately 79% nitrogen, 21% oxygen, 0.04% carbon dioxide, and a very small percentage of the noble gases, Ar, Ne, and Xe. Air, unless it is in a closed system, is in a constant state of flux, much more so than soil or water. Grab samples are transient and mark only a single timepoint, while time composite samples are necessary to represent of a period of time.

Pollutants in the air can exist in the gas phase, as is the case with the volatile organic compounds, which have low boiling points and evaporate readily at ambient temperature. The semivolatile compounds are often found in the liquid or solid state as part of an aerosol. Real-time sampling of air can occur, and the analytes introduced into the gas chromatograph can be detected immediately after sampling and separation. This can be accomplished using a sampling loop or even a syringe. If the analytes need to be concentrated to obtain lower detection, as is the case with most air methods, a large volume of air can be drawn in using a pump and passed through a cryogenically cooled trap or adsorbent. Once sampling has been completed, the trap can be heated to desorb the analytes into the gas chromatograph for separation. Air samples or gas samples can be collected and held until analysis. A container such as a SUMMA canister, a Tedlar bag, or a gas-sampling tube can be used to hold the sample in the gaseous state until analysis. In some methods the analytes will be separated from the air by trapping them on an absorbent material during sampling. The air is not stored; only the analytes in the air are kept for analysis. VOCs can be sampled by metering air

through Tenax or charcoal tubes. After sampling and trapping the analytes, they are thermally desorbed from the tubes by heating or extracted with a solvent prior to analysis by GC. SPME has also been used to sample analytes from air. The fiber is protruded and exposed to the surrounding air, and analytes are adsorbed into the polymeric coating on the fiber. Some mechanism, however, must be used to ensure that an accurate representation of the air or gas is in contact with the fiber during sampling.

Aerosols are defined to mean dispersions of any material in the liquid or solid phase in a gas stream or the atmosphere. Dusts, smoke, soot, mist, fumes, and fog are terms that can be used to describe aerosols under this definition (119). These aerosols can be sampled by passing the air through a filtering device that physically traps the liquid or solid particles that are present in the air. These filters can be as simple as a single piece of glass fiber filter paper or can be several inches thick, for example, the polyurethane foam (PUF) filters that are used for the sampling of SVOCs. When particles are sampled, the analytes of interest are often adsorbed onto the particles, and some method of liquid–solid extraction must be used to extract the analytes from the surface of the particles. Air can also be bubbled through a solution to trap the target analytes in a liquid using an impinger.

A large group of the USEPA methods for the determination of toxic organic compounds are collectively known as the TO series. Presently seventeen of these methods exist and are labeled as such, TO-1 through TO-17 (120,121). Table 15.18 summarizes 16 of these methods.

TABLE 15.18 USEPA Methods for Determination of Toxic Organic Compounds in Air

Method	Sampling Media	Target Analytes	Analytical Method
TO-1	Tenax tubes	VOCs: b.p. 80–200°C	GCMS
TO-2	Carbon molecular sieve tubes	VOCs: b.p. − 15°C to 120°C	GCMS
TO-3	Cryogenic trapping, real time	Nonpolar VOCs: b.p. −10 to 200°C	GCFID, GCECD, or GCPID
TO-4A	PUF cartridges—high volume	Pesticides and PCBs	GCECD, GCNPD, GCFPD, GCELCD, or GCMS
TO-5	Liquid impinger with dinitrophenylhydrazine	Aldehydes and ketones	HPLC/UV
TO-6	Liquid impinger with aniline	Phosgene	HPLC/UV
TO-7	Thermosorb/N cartridge	*N*-Nitrosodimethyl-amine	GCMS
TO-8	Liquid impinger with sodium hydroxide	Cresol/phenol	HPLC/UV
TO-9A	PUF cartridges	Polychlorinated dioxins and furans	HRGC/MS

TABLE 15.18 (*Continued*)

Method	Sampling Media	Target Analytes	Analytical Method
TO-10A	PUF cartridges—low volume	Pesticides and PCBs	GCECD, GCNPD, GCFPD, GCELCD, or GCMS
TO-12	SUMMA canisters	Nonmethane organic compounds—total organic carbon	FID only—no separation
TO-13A	PUF/XAD cartridges	PAHs	HPLC/UV or GC/FID or GCMS
TO-14A	SUMMA canisters	Nonpolar VOCs	GCMS
TO-15	SUMMA canisters	Polar and nonpolar VOCs	GCMS
TO-16	No sampling—field method	Atmospheric gases	Long, open-path FTIR monitoring
TO-17	Hybrid air tubes (Tenax GR, Carbopack B, Carbopack C Carbosieve SIII, Carboxen 1000)	VOCs	GCMS

Source: Adapted from References 120 and 121.

15.16.1 The Determination of Volatile Organic Compounds in Air Using Adsorbents and Gas Chromatography

When it comes to air analysis, the VOCs are the class of compounds that are of major concern simply because they naturally move to the gaseous state because of their volatility and low boiling points. They are more likely to be present in the air than the heavier pollutants such as the pesticides, PCBs, and other SVOCs. The VOCs can be sampled from air using a sampling pump and air tubes packed with an adsorbent that will trap VOCs. Much of the earlier discussion concerning the purge–trap and adsorbents (see Section 15.5.2) is relevant in this section also. USEPA Methods TO-1, TO-2, and TO-17 are methods for determining VOCs in air using tubes that are packed with a single absorbent or multiple absorbents. Such devices are called *air tubes* and will be referred to as such in future discussions. Method TO-1 uses Tenax, and Method TO-2 uses carbon molecular sieve (CMS) to trap different ranges of VOCs. Method TO-17 is a new and improved version of TO-1 and TO-2, which uses a variety of new generation sorbents (see Table 15.18), singly or in multisorbent packings, to target specific VOCs. All the air tube methods are very similar. Stainless-steel, nickel, or glass tubes, about 6 mm in diameter and 16.5 cm in length, are packed with the cleaned adsorbent, which is held in place by two plugs of glass wool or stainless steel screens. Air tubes can be packed in the laboratory or they can be purchased commercially. Many of the sorbents require extensive cleanup before they can be used to pack air tubes. For example, a Soxhlet extraction

using methanol and then pentane is recommended to clean Tenax before packing the tubes. The amount of sorbent placed in the tube will depended on the target VOCs and the characteristics of the sorbent. For example, CMS is used to trap more volatile VOCs than Tenax, and only approximately 0.5 g of CMS is needed to pack a normal air tube, while nearly 2–3 times as much Tenax is used. After packing, the tubes are also thermally cleaned by passing an inert gas, such as helium or nitrogen, through them for several hours while they are heated. For example, in TO-1, the Tenax tubes are heated to 275°C with a helium flow of about 100 mL/min for 4 h. CMS is thermally cleaned at 400°C for 16 h with a helium flow of 100–200 mL/min. A wide variety of adsorbents are commercially available, and prepacked tubes can also be purchased. After cleaning, the tubes are ready for sampling and are stored in sealed stainless-steel cylinders with a small amount of granular carbon to protect them from contamination. Extreme care must be taken to prevent the finished tubes from being contaminated by VOCs during shipping and storage. A tube that is unused in the sampling process and known as a "trip blank" is generally kept with each batch of tubes and accompanies the tubes on their sampling journey.

During sampling, the collection of a known and accurate volume of air is very important. Usually a small mechanical pump and a flow control device, such as a mass flow controller, are used to pass a metered amount of air through the tubes. Flows less the 100 mL/min are recommended for tube sampling. The sampling time will be dependent on the absorbent in the tube and the analyte. Each absorbate (analyte being trapped) has a breakthrough volume of air for that particular absorbent. The breakthrough volume is the amount of air that is required to pass the absorbate completely through a known weight of the absorbent. Some breakthrough volumes for some common VOCs on Tenax are shown in Table 15.19.

The breakthrough volume will be dependent on humidity, temperature, concentration of the analyte in the air being sampled, and the adsorbents in the air tube. The CMS used in TO-2 has a much higher breakthrough volume (usually >100 liters/gram) than does Tenax for the VOCs listed in Table 15.19. Usually a secondary tube is placed in series with primary sample tube to check for breakthrough during sampling. Much more information on sorbent characteristics and

TABLE 15.19 Breakthrough Volumes of Air on Tenax at 38°C

Compound	Liters/gram
1,1,1-Trichloroethane	6
Benzene	19
Toluene	97
Ethyl benzene	19
n-Heptane	20

Source: Taken from Reference 120.

breakthrough volumes for different VOCs on different sorbents can be found in Reference 121.

When the tubes are analyzed, the analytes can be desorbed thermally or displaced using a solvent. The disadvantage of using a solvent is the loss of sensitivity. Usually only 1 or 2 μL of solvent can be injected into to a capillary column. If several milliliters of solvent are required to desorb the tube, only a small fraction of the analytes on the tube is being introduced into the gas chromatograph. If the tubes are thermally desorbed, the analytes on the tube are quantitatively transferred to the gas chromatograph unless the sampling stream is split for some reason. In methods TO-1, TO-2, and TO-17 the tubes are thermally desorbed using a heating block or heater that fits around the sampling tubes. The sampling tubes are coupled into a gas stream using graphite or vespel ferrules and Swagelok fittings. Dry purging the air tubes with helium may be necessary if large of amounts of water have been collected on the tubes during the sampling process. Desorption occurs at an elevated temperature and depends on the properties of the sorbent and the analytes (e.g., ~350°C for CMS, 180°C for Tenax). The analytes are purged from the sampling tubes using helium at flows of approximately 30–50 mL/min for about 10 min. Because of the large desorption volume, the VOCs need to be refocused either cryogenically using a cooled trap that is filled with 60/80-mesh glass beads or another adsorbent (or a combination of both). During this process the trap is vented to the air. Liquid nitrogen or oxygen are typically used to cool the trap. After purging, the trap is placed in line with the GCMS (or gas chromatograph with alternate detector) and rapidly heated. The carrier-gas flow is used to transfer the contents of the trap to the head of the analytical column. Because low temperatures are needed to focus extremely light VOCs at the head of the column, the oven is often cryogenically cooled to—70°C to start the temperature program. The technique of cryofocusing can be used instead of cooling the entire oven, which requires a large quantity of liquid nitrogen. Cryofocusing can be accomplished by cooling a short section (~10 cm) of the uncoated fused silica that is connected to the column with liquid nitrogen. This saves a great deal of liquid nitrogen, but colder temperatures are required for very volatile compounds. The gas chromatographic separation of the VOCs in TO-1 and TO-2 is performed on a 50–60-m column with either a 0.25- or 0.32-mm diameter column. The stationary phase must also be able to withstand the cold temperatures if cryogenic cooling of the oven is used. Often a DB-1 or DB-5 stationary phase is used to separate the VOCs.

Standards for calibration can be prepared in a number of ways when using air tubes. The VOCs can be spiked onto the absorbent in the tube using a microsyringe and a methanolic solution of the VOCs. Gas standards that contain the VOCs in air or nitrogen can be purchased or mixed. A metered volume of the standard is allowed to flow through the tubes to achieve the desired calibration level. Another alternative is to use gas permeation tubes (see Chapter 8), which allow the VOCs to diffuse into the sampling stream at a controlled rate through a plastic tube. Permeation tubes are calibrated gravimetrically, with the weight loss

of the tube equated to the weight of the escaping material. A constant temperature control must be maintained on the tubes (122).

When reporting VOC concentrations in air using air tube methods, the VOCs are often reported as weight per unit volume of air at 25°C and one atmosphere of pressure. The volume of air collected during sampling must be corrected to these conditions using the ideal-gas laws, therefore, the temperature and atmospheric pressure must have been recorded during the collection of the air sample. Sometimes units of volume are used and the contaminants are reported as part per million by volume (ppmv) or part per billion by volume (ppbv) in the air. When the assumption is made that all gasses are ideal, units of μmole/mole can be used. For example, the unit of μmol/mol of air is equivalent to ppmv. Air analysis using air tubes has several inherent weaknesses. Artifact peaks originating from the air tubes through either contamination or breakdown of the adsorbent at elevated temperatures are common (123). Tenax, for example, can also break down to form benzenaldehyde, phenol, acetophenone, dibutyl phthalate, and other unidentified unknowns when exposed to ozone and nitrogen dioxide during sampling (124). The breakthrough volume can easily be underestimated at a sampling site because of humidity or high analyte concentrations (125). A tube sample can be desorbed only once. There is no room for reinjection or even dilution without resampling. Preparing the tubes and handling the tubes is also very labor-intensive unless commercially packed tubes are purchased, but they still require thermal cleaning. They also must be checked for cleanliness prior to use.

15.16.2 The Determination of Volatile Organic Compounds in Air Using SUMMA Canisters and GCMS with Cryogenic Trapping

SUMMA canisters are stainless-steel spheres that have a passivated stainless-steel interior that is inert to VOCs and allow for long-term storage of air. The canisters come in a variety of sizes; the most common size is 6 L, and they have a needle valve that allows for sampling and removal of air. SUMMA canisters that are coated internally with fused silica have become available. USEPA methods TO-14A and TO-15 describe the determination of VOCs in air using the SUMMA canisters. TO-15 is a new version of the canister method and has an expanded target list with a total of 97 VOCs that include some of the more polar and water-soluble VOCs. Method TO-15 establishes method performance criteria for acceptance of data that allows for the use of alternate but equivalent sampling equipment. TO-15 inclusively uses GCMS and has enhanced quality assurance provisions.

SUMMA canisters are cleaned by alternatively filling them with humidified air, venting them, and then pulling a very low vacuum (<0.05 mm Hg) for a total of three cycles. Sometimes the canisters require heating during the process to remove less volatile compounds. On the last cycle they are filled with humidified air, and each canister must be analyzed to certify that it is clean of VOCs. A pressurized leak test is also required. The cleaned canisters are then evacuated and ready for sampling.

Subatmospheric or pressurized sampling can be used. When subatmospheric sampling is used, a critical orifice or similar flow control device is used to control the flow of air into the canister when it is opened to the air. The flowrate of air into the canister will determine the sampling period. Canisters can be filled to within 1 or 2 psi of ambient pressure. The maximum volume of air that can be collected will be slightly less than the volume of the SUMMA canister. A metal-bellows-type pump is used to draw air into the SUMMA canister during pressurized sampling. Because the canister can be pressurized, larger volumes of air can be collected, and longer sampling periods are possible. If a 6-L SUMMA canister is set to collect air at 10 mL/min in the subatmospheric mode, the maximum sampling period will be approximately $8\frac{1}{2}$ hours. However, with a pump, the sampling period can be easily extended to 24 h at the same flowrate. A mechanical pump, however, must be available for each sampling point and each pump must also be certified to be clean of VOCs. Temperature and pressure readings are required during the sampling process. Often the flowrate at the beginning and end of the sampling process is taken to ensure that an accurate sampling has occurred.

Prior to analysis the pressure of the SUMMA is measured and often humidified zero air is added to the canister to perform a twofold dilution if subatmospheric sampling was used in collecting the sample. Humidified zero air is prepared by bubbling nitrogen gas or air through a container of distilled water. It is imperative to demonstrate that this airstream is free from any VOCs. This humidified zero air is extremely important in the analysis of the SUMMA canisters because it used for the method blank, in the cleaning process, and to demonstrate that the analytical instrumentation, sampling equipment, and SUMMA canisters are free of contamination. The air is humidified because of the film of water it creates on the inside of the SUMMA canisters and along the flow paths of the sampling devices, equipment, and instrumentation. This film of water aids in the transmission of the VOCs through the plumbing of the analytical system.

The analytical system consists of two components: an automated cryogenic preconcentrator and the GCMS. Manual cryogenic concentration of air samples is possible but is very tedious and time-consuming and not as reproducible as with automated systems. A number of commercial systems are available and are listed in TO-15 (121). During analysis the canisters are connected to a manifold (some can hold up to 20 canisters) and the air is pulled by a mechanical pump through an electronic mass flow controller (EMFC) that accurately controls the flow and volume of air that is delivered to the primary trap. The EMFC measures gas flows at standard temperature and pressure (STP, 0°C, and 1 atm). Normally 250–500 mL of air at STP is sampled at a constant flowrate between 50 and 150 mL/min. A variety of trap packings and trap temperatures can be used during the cryogenic preconcentration step. If glass beads are used, a very cold temperature ($\leq$ -150°C) must be maintained on the primary trap using liquid nitrogen, argon, or oxygen. Trapping can occur at ambient temperatures if the trap is packed with adsorbents. One of the primary traps listed in TO-15 is filled with Carbopack B (200 mg) and Carbosieve S-III (59 mg). This trap has been

found to retain the VOCs and allow a certain amount of water vapor to pass through at ambient temperatures (126). During sample introduction, water vapor must be removed from the air sample in some manner. Water can freeze and plug cryogenic traps or cryofocusing units. If water reaches the mass spectrometer, it can prevent ionization and suppress the signal from the mass spectrometer. Water vapor can be removed using a Nafion dryer as described in TO-14, but this precludes the analysis of polar VOCs. The Nafion dryer is a length of tubing containing an inner tube made from a water permeable membrane (Nafion). Nitrogen gas flows through the outer tube and over the inner tube, which carries the air sample, stripping away the water vapor that passes through the Nafion. Other methods of water management are used to allow for the determination of polar VOCs.

After the air sample has been loaded onto the primary trap, it is heated and the VOCs are transferred to either a secondary trap or a cryofocusing device for refocusing. In some configurations the primary trap will be purged directly onto the head of a capillary column that is cryogenically cooled.

An application of the TO-15 method for the analysis VOCs using such a technique is described below. In this application three cryogenically cooled traps are used. This three stage flow path and transfer is shown in Figure 15.24.

Module 1 contains a glass bead trap (60/80 mesh) that is cooled to -150°C while the air sample is passed through at 100 mL/min. After 250 mL of air has been loaded on the primary trap, module 1 is heated to 5°C and the VOCs are purged onto module 2 with a helium flow of 15 mL/min for 5 min. The small amount of water ($\sim$5–10 μL) that was present in the air sample is in the liquid state and remains on the glass beads while the VOCs are purged to the secondary trap, which is filled with Tenax and cooled to -20°C with cryogen. After 5 min the primary trap is vented and baked while the secondary trap is desorbed at 180°C for 2 min with the carrier flow set at 2 mL/min. The VOCs are desorbed from the secondary trap and cryofocused to a narrow band on a short length of capillary fused-silica tubing (silcosteel, 0.53 mm i.d.) that is cooled to -160°C with liquid nitrogen. After the secondary trap has been desorbed, it is vented and baked. The cold section of fused-silica tubing is then heated rapidly with a flow of hot nitrogen gas to release the trapped VOCs onto the chromatographic column for separation. The carrier-gas flow is also reduced to 1.0 mL/min at this time. The transfer lines and valves in the preconcentrator are heated to 80°C to prevent condensation of the VOCs. Most of the lines are made of nickel tubing, which, when moistened with the humidified air, is an inert surface for transmitting the VOCs along the flow path of the preconcentrator. An example total ion chromatogram of an air standard containing 82 VOCs at 50 ppbv is shown in Figure 15.25.

The GCMS is calibrated using five levels of air standards over the range 1–50 ppbv. When VOC stock standards are purchased commercially, they are contained in compressed-gas cylinders at high pressure and can be used for several years. Stock standards are often made with the concentration of the VOCs at approximately 10 ppmv. Calibration standards are prepared by blending or

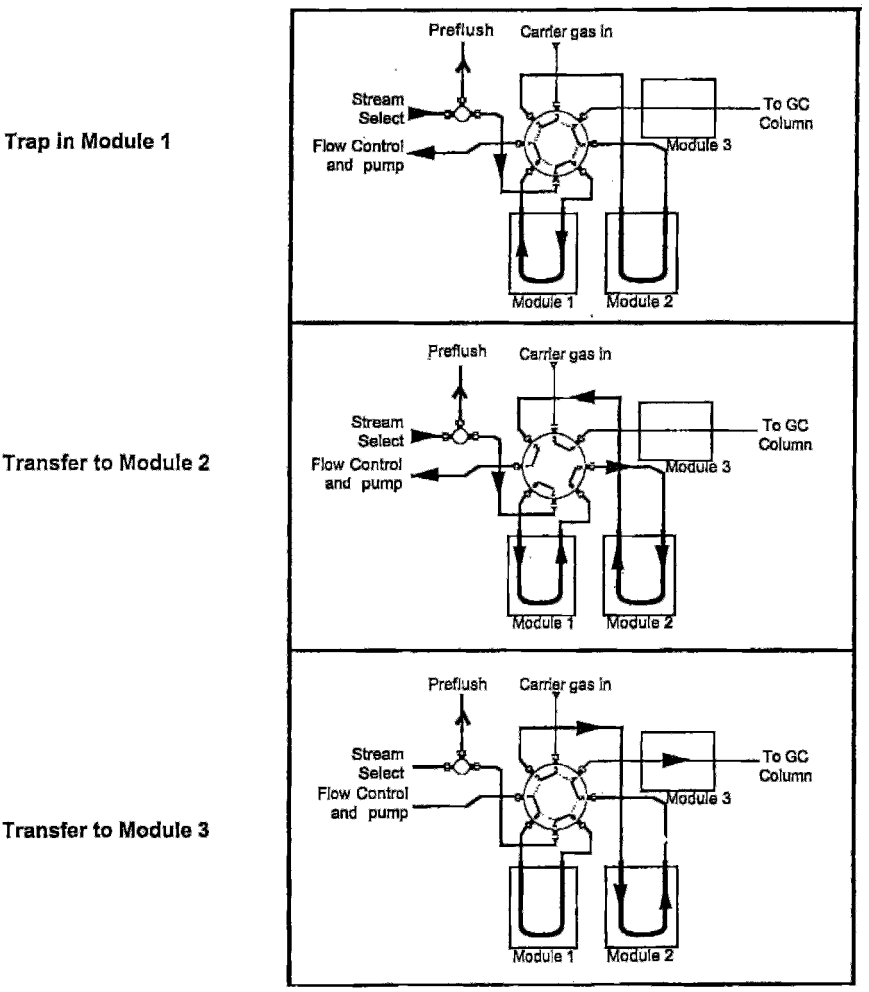
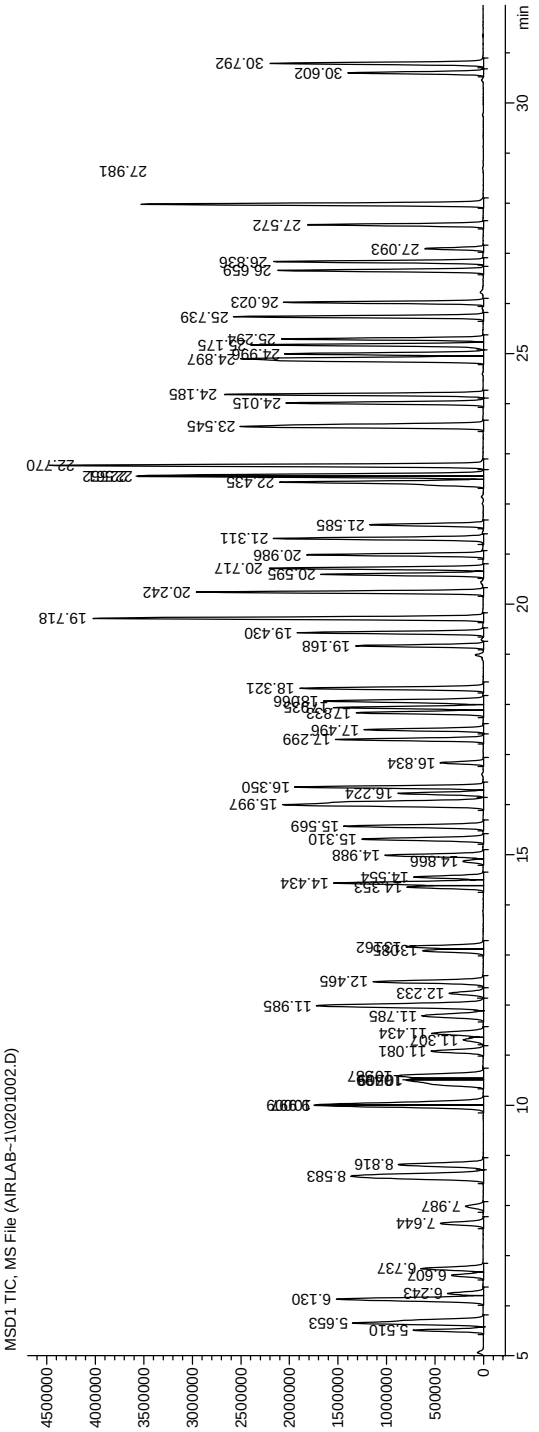


FIGURE 15.24 Three-stage flow path for the cryogenic preconcentration of VOCs from air and the removal of water vapor by microscale purge and trap, Entech Technologies, Inc.

dynamically diluting a stock standard mixture with zero air using a mixing manifold and EMFCs to obtain the desired concentration. A schematic diagram of a standard mixing manifold is shown in Figure 15.26. Standard mixtures can be prepared and stored in SUMMA canisters, or the blended gas stream can be sampled directly from the manifold with the cryogenic preconcentrator. Individual standards can be prepared for each concentration level or the volume that is sampled can be adjusted to produce the needed amount. For example, if 250 mL is the nominal volume that is sampled, then taking 50 mL of a 10-ppbv standard is equivalent to loading 250 mL of a 2-ppbv standard. Very small sample volumes, however, cannot be measured accurately by the EMFC because


MSD1 TIC, MS File (AIRLAB~1\0201002.D)
min
5.510
5.653
6.130
6.243
6.602
6.737
7.644
7.987
8.583
8.816
11.081
11.307
11.434
11.785
11.985
12.233
12.465
14.352
14.434
14.554
14.866
14.988
15.310
15.569
15.997
16.224
16.350
16.834
17.299
17.496
18.321
19.168
19.430
19.718
20.242
20.595
20.717
20.986
21.311
21.585
22.435
23.545
24.015
24.185
24.897
25.294
25.739
26.023
26.659
27.093
27.572
27.981
30.602
30.792

Compound	RT (min)	Compound	RT (min)	Compound	RT (min)
Bromochloromethane	14.86	1,1-Dichloroethane	13.08	Tetrachloroethene	20.72
1,4-Difluorobenzene	16.83	Vinyl Acetate	13.16	2-Hexanone	20.98
Chlorobenzene d5	22.38	cis-1,2-Dichloroethene	14.35	Dibromochloromethane	21.31
Propene	5.51	2-Butanone	14.43	1,2-Dibromoethane	21.59
Dichlorodifluoromethane	5.64	Ethyl Acetate	14.44	Chlorobenzene	22.44
Chlorodifluoromethane	5.71	Methyl Acrylate	14.55	1,1,1,2-Tetrachloroethane	22.58
Freon 114	6.13	Chloroform	14.98	Ethylbenzene	22.55
Chloromethane	6.25	1,1,1-Trichloroethane	15.31	m/p-Xylene	22.77
Vinyl Chloride	6.60	Carbon Tetrachloride	15.57	o-Xylene	23.54
1,3-Butadiene	6.73	1,2-Dichloroethane	16.23	Styrene	23.58
Bromomethane	7.65	Benzene	16.05	Bromoform	24.02
Chloroethane	7.99	Isooctane	16.00	Cumene	24.18
Dichlorofluoromethane	8.55	Heptane	16.34	1,1,2,2-Tetrachloroethane	24.86
Trichlorofluoromethane	8.60	Trichloroethene	17.30	1,2,3-Trichloropropane	25.00
Pentane	8.81	Ethyl Acrylate	17.50	Bromobenzene	24.91
Acrolein	10.01	1,2-Dichloropropane	17.83	4-Ethyltoluene	25.17
1,1-Dichloroethene	10.04	Methyl Methacrylate	17.93	1,3,5-Trimethylbenzene	25.30
Freon 113	10.00	Dibromomethane	18.07	Alpha Methyl Styrene	25.74
Acetone	10.42	1,4-Dioxane	18.04	1,2,4-Trimethylbenzene	26.02
Methyl Iodide	10.50	Bromodichloromethane	18.32	1,3-Dichlorobenzene	26.66
Carbon Disulfide	10.60	cis-1,3-Dichloropropene	19.17	1,4-Dichlorobenzene	26.84
Acetonitrile	11.31	4-Methyl-2-Pentanone	19.43	Benzyl Chloride	27.10
3-Chloropropene	11.08	Toluene	19.73	1,2-Dichlorobenzene	27.57
Methylene Chloride	11.43	Octane	19.72	Hexachloroethane	27.98
tert-Butyl Alcohol	11.79	trans-1,3-Dichloropropene	20.24	1,2,4-Trichlorobenzene	30.60
Acrylonitrile	12.23	Ethyl Methacrylate	20.25	Hexachlorobutadiene	30.79
Trans-1,2-Dichloroethene	12.00	1,1,2-Trichloroethane	20.59		

FIGURE 15.25 VOCs in air by GCMS using Method TO-15. Conditions: injector 150°C; carrier gas, helium 1.0 mL/min; column, 60-m × 0.32-mm × 1.8- μm film; oven, 35°C for 4.5 min, and ramp to 60°C at 4°C/min, ramp to 220°C at 8°C/min and hold for 2.5 min. Direct interface 280°C; MSD, EI 70 eV and scanned 35–300 amu; tune, BFB. (Courtesy of Lancaster Laboratories, Air Laboratory.)

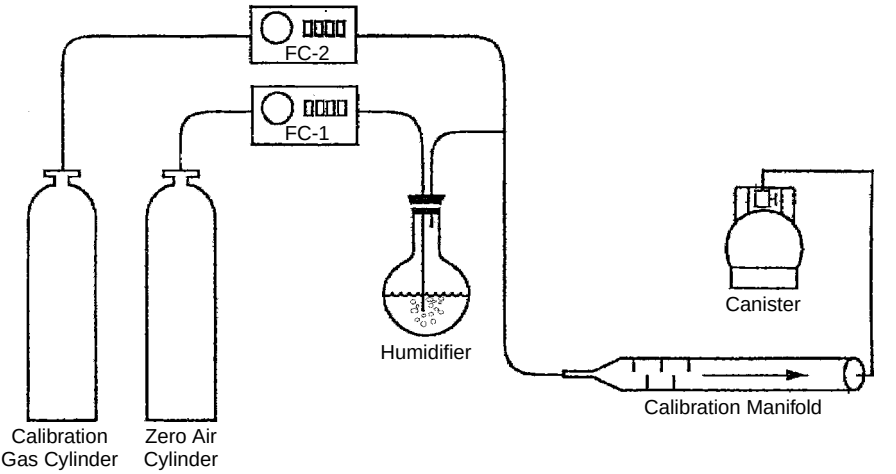


FIGURE 15.26 Mixing manifold used to blend VOC working standards in air for TO-14 calibration; metered blending of calibration stock standard and zero air accomplished through electronic mass flow controllers (FC-1 and FC-2) (adapted from Reference 121).

of the short sampling times involved. Air standards can also be produced by other techniques. Static dilution can be used to produce air standards containing VOCs. Measured volumes of VOCs are injected into heated glass, gasdilution bulbs that are a known volume (usually 1 or 2 Ls). The VOCs are then serially diluted using more heated gas bulbs and syringes. A portion of the final dilution is injected into a metered gas stream to produce the desired concentration. VOCs contained in aqueous standards can also be purged into a gas stream to produce air standards at the desired concentrations (127). Permeation tubes (121) or diffusion devices (128) can be placed in a metered gas flow to produce the necessary VOC standards (122) (also see Chapter 8). Regardless of the method of standard preparation, the use of SUMMA canisters and cryogenic preconcentration enables a detection limit of 0.2 ppbv for most of the VOCs in air.

15.16.3 The Determination of Semivolatile Organic Compounds in Air

Methods for the analysis of SVOCs in air involve the entrapment on a filtration device or absorbent or in a liquid using an impinger. The gas chromatographic techniques that are used for their determination will depend on the target compounds and are often the same as those used for their determination in soil and water. Semivolatile compounds that are produced by combustion processes are of particular concern. The PAHs head the list and have received a great deal of interest. In fact, over 40% of the environmental applications appearing in the literature from 1999 to 2001 dealt with the analysis PAHs (7). In the traditional approach using USEPA Method TO-13A, air samples (typically 300–400 m^3 over 24 h) are drawn through a quartz filter that is backed up

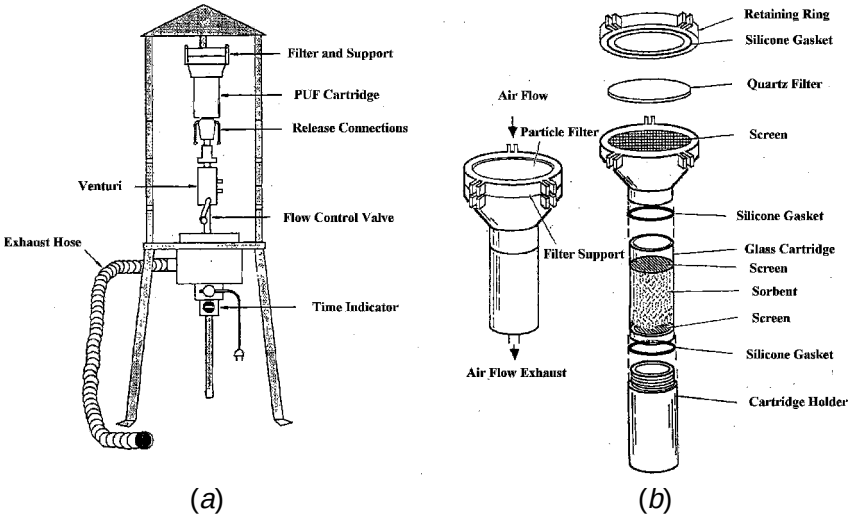


FIGURE 15.27 (a) High-volume sampler for collection of semivolatile organic compounds from air; (b) schematic diagram of air filter assembly containing quartz filter and polyurethane foam (PUF) cartridge (adapted from Reference 121).

by an absorbent of either polyurethane foam (PUF) or XAD-2 resin using a high volume pump. This sampling configuration is shown in Figure 15.27. The PAHs span a vapor pressure of 1.1×10^{-2} to 2×10^{-13} kPa. Those that have a vapor pressure greater than 10^{-8} kPa will be distributed between the vapor phase and particulate phase and, therefore, will be trapped on both the filter and absorbent. Naphthalene breaks through PUF rather easily so XAD-2 is recommended when lighter PAHs are to be determined. PUF is much easier to handle and cleanup than XAD-2, however. Extensive effort to clean up both PUF and XAD-2 resin sampling cartridges is required before they can be used for field sampling. A 16-h Soxhlet extraction using acetone is required for PUF and two 16-h Soxhlet extractions using methylene chloride is required for XAD-2 resin. After sampling the filters and absorbent are extracted using a Soxhlet extraction with methylene chloride (for XAD-2 optional) or diethyl ether. Extracts are cleaned up with silicagel and concentrated to a volume of 1.0 mL. The PAHs are separated and detected using GCMS using instrument conditions that are very similar to USEPA SW8270 described above (14,125). An internal standard calibration over the range 0.5–2.5 ng/μL is used with five internal standards: naphthalene-d_8, acenaphthene-d_{10}, phenathrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. Two field surrogate standards, fluoranthene-d_{10} and benzo (a) pyrene-d_{12} are spiked onto the PUF cartridges in the field prior to sampling and two laboratory surrogates, fluorene-d_{10} and pyrene-d_{10}, are spiked in during the Soxhlet extraction. A number of blanks are required. A laboratory method blank (LMB) is required to monitor for possible laboratory contamination. An unused filter–cartridge assembly is extracted and analyzed. A field blank consisting of

a filter/cartridge assembly, which was sent to the field with the samples and had no air drawn through it, must also be extracted and analyzed. A process blank for each batch (20) of filters and cartridges prepared in the laboratory must be also analyzed. A solvent blank, using only the solvents prescribed in the method and no filter or cartridge, must also be carried through the procedure and analyzed. PAHs are reported in units of ng/m^3 of air and converted to ppbv using the ideal-gas law if desired (121).

Pesticides and PCBs can be sampled in the same manner as the PAHs. In Method TO-4A, large volumes of air sample (300–400 m^3) are drawn through a quartz filter and a PUF cartridge as shown in Figure 15.27. After sampling and adding the appropriate surrogate standards, the filter and PUF cartridges are extracted with 10% diethyl ether–hexane using a Soxhlet apparatus for a minimum of 16 h. The quartz filters and PUF cartridge assemblies are extracted. Cleanup is optional and dependent on the target list of compounds. The cleanups available are the same as those used for the analysis of water and soil samples. After solvent evaporation, the organochlorine pesticides and PCBs are determined using GCECD or GCELCD. The organophosphorus pesticides are determined by GCNPD or GCFPD. GCMS can also be used, but detection limits are typically higher. Detection limits of 0.001–50 $\mu g/m^3$ are possible and dependent on the analyte and the sampling period. Prior to use the filter assemblies must be certified clean and field and method blanks are also required. Method TO-10A has the same target compounds (pesticides and PCBs) and follows the same analytical procedures except that it incorporates a low-volume sampling pump that holds sampling tubes, which hold layers of PUF and Tenax. It can be used for indoor or outdoor air, whereas the previous high-volume methods, described above, are used almost exclusively for outdoor air (121).

Polychlorinated and polybrominated dibenzo-*p*-dioxins and dibenzo-*p*-furans, which are byproducts of combustion, can also be sampled in air using the high-volume air sampler and the quartz filter and PUF cartridges depicted in Figure 15.27. The dioxins and furans are determined by high-resolution GCMS. Detection limits of 10–20 fg/m^3 are possible for these compounds in air when 300–400 m^3 of air is sampled (121).

15.17 HANDLING OF GAS CHROMATOGRAPHIC DATA IN ENVIRONMENTAL ANALYSIS

Every field of analytical chemistry has its own unique methods in evaluating and crunching data to produce the final reported number. Environmental analysis is no different, especially in the reporting of regulatory data. The USEPA methods have a number of guidelines and specifications that must be met in order to ensure the quality and integrity of the data. Many of the USEPA methods have performance criteria that must be met before samples can be analyzed. Method 8000 from USEPA SW846 is not a specific method for the determination a specific target list of compounds, but rather it provides guidance on analytical chromatography,

describes calibration procedures, and lists quality control requirements common to all the gas chromatographic methods in USEPA SW846 (13).

15.17.1 Quantification

Quantification begins with the calibration that can be either an external or internal standard calibration. The GCMS methods use internal standards that are often stable, isotopically labeled analogs of specific target compounds. Gas chromatographic methods that employ other detectors often use internal standards that are brominated or fluorinated, but resemble the target analytes. When internal standards are used, the USEPA methods usually require that the internal standard peak areas be 50−150% of the internal standard areas of a selected calibration standard or continuing calibration standard. This is to ensure that a full-measure injection has been made and that the gas chromatograph and detector are operating correctly. When complex samples are analyzed using detectors other than a mass spectrometer, internal standard calibration may not be a good idea, because of the possibility that an interfering peak will coelute with the internal standard. An internal standard calibration, however, is usually more accurate, because the internal standard compensates for small fluctuations in injection volume and changes in detector response. A minimum of five levels of standard are required for nearly all USEPA methods and should define the working range of the detector or correspond to the actual range of concentrations found in samples. The lowest concentration for each analyte should be at the limit of quantitation (LOQ) for the analyte. When the calibration is completed, a relative response factor (RRF) or calibration factor is calibrated for each calibration level for each analyte. If the relative standard deviation (RSD) of these response factors is less than method-defined percentage the calibration is assumed to be linear, the y intercept is assumed to be zero, and the average response factor of the five levels can be used for quantification of samples. Method 8000 sets this RSD at 20% and this is typical for gas chromatographic detectors other than the mass spectrometer. The methods using GCMS, Methods 8260B and 8270C, have a lower limit of 15% for the RSD (13). If the RSD is greater than this limit, then a linear regression is required; the correlation coefficient of the linear regression must be greater than 0.99 for the calibration to be acceptable. Nonlinear calibrations are permissible using polynomial or quadratic fits.

The GCMS methods designate a number of compounds as system performance check compounds (SPCCs). The SPCCs are compounds that show poor chromatographic performance when the analytical system or the standards begin to deteriorate. These compounds must have a response factor that is greater than a value defined in the method. For example, Method 8260B lists five SPCC compounds, chloromethane, 1,1-dichloroethane, bromoform, chlorobenzene, and 1,1,2,2-tetrachloroethane, that must have average RRFs greater than 0.10, 0.10, 0.10, 0.30, and 0.30. If any of these compounds show lower response factors, the initial calibration is to be considered invalid. The GCMS Method 8270 for

SVOCs lists four compounds, *N*-nitroso-di-*n*-propylamine, hexachlorocyclopentadiene, 2,4-dinitrophenol, and 4-nitrophenol. These compounds must show a response factor greater than 0.05.

The initial calibration may be used to quantify samples as long as the instrument response remains stable for each analyte. A continuing calibration standard is usually injected every 12-h time period. USEPA SW846 Method 8000 allows the response of an analyte to drift ±15% before recalibration or corrective action is necessary, although other methods may have different specifications.

When the initial calibration fails or the continuing calibration does meet the specifications for an analyte or group of analytes, maintenance on the gas chromatograph is often required. Environmental samples are often dirty and deposit nonvolatile residues in the injection port and on the front end of the column. A well-maintained/functioning analytical system is essential for high-quality data. Maintenance on the gas chromatograph involves such tasks as removing the injection liner and replacing it with a clean, deactivated one or rinsing it with solvent. Often the inside of the injection port requires cleaning, which can be accomplished by wiping it with a swab soaked in solvent or completely rinsing it with solvent. Clipping a short section from the front of the column (0.5–1 m) or the guard column may be necessary to restore response or peak shape to polar analytes. Whenever maintenance is done on the injector it is a good practice to change the septum also. After completion of front-end maintenance, the gas chromatographic oven is often set to bake for several hours before recalibrating.

Chemical standards are essential for calibration and the spiking of samples. Normally stock standards need to be made for each analyte by weighing the pure compound into a volumetric flask and diluting them to volume with solvent. Typically stock standards are prepared at approximately 1000 μg/mL. The stock standards are combined and further diluted to make intermediate mixtures containing a number of compounds, which are then further diluted to produce the calibration standards. Maintaining and preparing standards can be a very time-consuming and a tedious task, especially for some methods that list over 100 target compounds. Accurate records and documentation listing the source, purity, lot number, and expiration date for each standard must be maintained. Many of the USEPA methods list storage conditions and expiration periods for standards. Storage times of 6 months to a year are typical for stock standards if they are stored in a freezer (ca. −10 to −20°C) and protected from light. Calibration standards are usually prepared weekly and stored in a refrigerator when not in use. Many laboratories purchase intermediate standards from commercial suppliers that can supply certification for the standard. Ideally a standard should be traceable to a certified reference standard from the National Institute of Standards and Technology (NIST). Second source verification of a standard is a good practice and provides confirmation of compound identity, stability, and purity. When new standards are made, comparison of compound responses and retention times to those of the old standards can catch mistakes in sample preparation and add assurance that the new standards are acceptable. Second-source

verification is practiced when matrix spiking solutions are made from a different source of standards than those used in the method.

Quantification is typically based on area, although peak height can be used successfully in complex samples that contain a multitude of peaks in the chromatogram. When computer data systems are used, care must be taken to ensure that the analog-to-digital converter takes enough samples across the peak to accurately define the peak. Ideally 10–14 points across the peak are needed to adequately define its shape. Good peak shape is essential for accurate quantification. Tailing peaks are difficult to integrate accurately and compounds that demonstrate tailing generally have diminished and nonlinear response at the lower end of the calibration curve. Active sites in the gas chromatograph can be minimized by the following practices:

1. Correct column installation of both the injector and detector ends.
2. Clean and deactivated or silanized injection liners.
3. Sharp, square cuts of capillary columns.
4. Maintaining a pressure tight and oxygen-free system since oxygen can oxidize the stationary phase of heated columns.
5. Proper injection port maintenance by frequently changing septum, solvent cleaning metal surfaces, and removing fragments of septum.
6. Periodically clipping the front end of the analytical column or guard column that has been fouled with nonvolatile residue.

15.17.2 Qualification

In gas chromatography peaks are identified by their retention times. Analyte retention times are determined during calibration by injecting standards of the analytes. Stable retention times are a prerequisite to proper identification of analyte peaks in the chromatogram and the prevention of falsely identifying artifact peaks. This means that the chromatograph must be free of leaks and have precise temperature and pneumatic control of the carrier-gas flow. Absolute retention times are used when no internal is present. Normally retention time windows are established for each target analyte and surrogate standard on the compound list. According to USEPA Method 8000, retention time windows can be established by injecting each standard mixture at least 3 times spaced over a 72-h period. The retention times for each analyte are measured to three decimal places, and the standard deviation(s) of the retention times is (are) multiplied by 3 to determine width of the window (this corresponds to approximately the 95% confidence interval around the mean). The retention time window is, therefore, defined as ± 3 s around the retention time of the analyte. If the standard deviation is 0.000, which is quite possible with modern gas chromatographs, then an absolute window of ± 0.003 min is used (13). Although the absolute retention time may change because of minor adjustments to the carrier flow or clipping of the column during maintenance, this retention time window can still be applied to the updated retention time. Practically speaking, however, a good analyst will through experience

set the retention time windows depending on the instrument and column that is being used. Very narrow windows may be necessary for some methods, such as PCB congener analysis, and possible if the chromatograph is equipped with electronic pressure control. Narrow windows, however, can preclude hits for target analytes, especially if an older-model gas chromatograph is being used. On the other hand, setting the retention time windows too wide will create many false positives. Most of the USEPA methods contain surrogate peaks, which can aid the analyst in recognizing drifts in retention times between calibrations. When dual columns are used, which is the practice in the majority of methods using detectors other the mass spectrometer, retention time windows are established for each column. If a target analyte falls within the retention time window on the primary column, then, to be confirmed, it must be present in the retention time window on the second column at approximately the same concentration. If a disparity exists between the concentration on the primary and secondary columns, it is possible that an artifact peak is coeluting with the target analyte on the column with the largest peak. It is also possible, especially on a busy chromatogram, that chance has allowed an artifact peak to fall into one or both of the retention time windows. Peaks identified as target analytes by gas chromatographic methods can also be confirmed by GCMS rather than using a second column. Unfortunately, the mass-selective detector is not as sensitive as a number of the detectors, which are commonly used in environmental analysis (e.g., ECD). The sample extract often has to be further concentrated by evaporating more of the solvent, and the gas chromatographic conditions must be transferred to the GCMS to locate the peak of interest. Often confirmation by GCMS cannot be accomplished. Absolute retention times for each compound are established in the initial calibration and updated when continuing calibration verification standards are injected. Retention windows are usually valid until the column is changed, and then new ones must be generated.

Peak identification for target analytes in GCMS analysis is based on relative retention times (RRTs) of the analyte compared to the designated internal standard and the mass spectrum of the analyte. Methods 8260B and 8270C allow a RRT window of ± 0.06 min. The second qualification is based on the mass spectrum of the target analyte. The characteristic ions in the spectrum of an analyte must maximize in the same scan (± 1), and the characteristic ions present in the suspected target peak must be within $\pm 30\%$ of the relative abundances of these ions in the reference spectrum (usually generated from a midlevel standard). When the retention and spectral qualification are met, the peak can be considered a "hit" for that target analyte. The luxury with the MSD is that unknown peaks that do not qualify as target compounds can be searched against EI spectral libraries and tentatively identified. For proper qualification of mass spectral data, the MSD must be tuned properly. Mass assignment must be accurate and cannot drift during analysis; otherwise the correct quantification ions will not be integrated and target compounds will not be detected. Peak widths of ions are usually set to approximately 0.5 amu at half-height so that unit resolution of atomic mass is

possible on benchtop mass spectrometers. The mass spectrometer cannot differentiate between structure isomers. Since these must be identified using retention time, Methods 8260B and 8270B require that valley between the structural isomers when compared to the peak height be less than 25%. One exception to this rule is the meta and para isomers of xylene that are reported as one unity because they are rarely separated.

15.17.3 Quality Assurance and Control

Quality assurance (QA) and quality control (QC) are terms that are often used interchangeably, much to the chagrin of the purists that work in these areas and enforce these concepts. *Quality assurance* is a global and administrative term. In the environmental laboratory, it refers to the programs and systems that are in place to ensure data is being produced in the correct manner using the proper methods and techniques and defines the measures, or quality control, that will be used to monitor that this process is occurring. The QA program in a laboratory is often defined by its quality assurance plan (QAP) that contains standard operating procedures (SOPs) for maintaining and monitoring quality in the laboratory. Often QAPs are project specific and are written by outside sources for a specific environmental project. When this occurs, the QAP at the laboratory and the project QAP must be reconciled before sample analysis can begin. Often laboratories will be required to follow the project QAP for the duration of the project rather than their own.

Various quality control checks are define in the USEPA methods dealing with gas chromatography to control errors and ensure the methods are being run in the proper manner. In environmental analysis, nobody knows the correct answer unlike in some industries (e.g., food and pharmaceutical) where a target value is confirmed or denied. Environmental data can be and is often compared and, therefore it is important that methods be run in a controlled manner. Results are often far less than quantitative. Precision and accuracy are monitored, but in ways that are different from those in other fields of analytical chemistry. These concepts will be addressed below.

15.17.3.1 Initial Demonstration of Proficiency (IDPF)

Prior to running a method, an analyst is often required to demonstrate proficiency in analyzing samples using the method. Many of the USEPA methods require this initial demonstration, and often project QAPs require this for a specific analysis. Sometimes special methods are developed in laboratories for several specific compounds that cannot be determined by routine methods and an IDPF is required. In the IDPF the analyst is required to spike four samples in the appropriate matrix (usually water, soil, or air) and analyze them according to the method. Usually the target analytes are spiked into a clean matrix such as laboratory reagent water or sand to minimize matrix effects. Accuracy is monitored by recoveries of the analyte and precision by the RSD. Often acceptance windows for accuracy and precision are defined in the method or the QAP. These studies

are often referred to as "quad studies," in the language of the environmental laboratory. If the accuracy and precision requirements are met, the analyst (or sometimes the entire laboratory) is deemed proficient and the analysis of samples by the method can begin. Recoveries of 70 to 130% are usually acceptable. Records of quad studies are kept in the laboratory and are often requested by QA auditors.

15.17.3.2 Surrogate Standards (SSs)

Most of the USEPA methods require the use of surrogate standards or surrogates. A surrogate is a nontarget analyte that is added to a sample prior to the extraction. Surrogates normally have similar chemical structures to the target analytes and in some methods as many six surrogate standards are used. In GCMS analysis the surrogates are often deuterated analogs of target compounds. In other GC methods, surrogates are often brominated or fluorinated to set them apart from target analytes. Naturally the surrogates must separated from the other target analytes in the sample. The surrogates monitor the efficiency of the extraction, the cleanup, and evaporation of the solvent if they have been used on a sample. Acceptable recovery limits are given in the methods. If a surrogate recovery is not within the acceptance window the sample must be reanalyzed. Surrogate recoveries can be used to troubleshoot problems with the analytical method or they can be used to demonstrate that the method is unsuitable for analyzing a particular sample matrix. Unfortunately, low-quality laboratories often erroneously blame poor surrogate recoveries on sample matrix effects, when improper sample preparation or instrumental problems are to blame for low recoveries of surrogates.

15.17.3.3 Method Blanks (MBs)

Typically a method blank is required to be analyzed daily or with each batch of 20 samples. The method blank is treated like any other sample except that a clean material is used. Surrogate standards are added to the method blank. Aqueous blanks are usually deionized or reagent water. Soils may consist only of the sodium sulfate used to dry the soil, or sometimes, clean sand is used. In air analysis the method blank is usually humidified zero air. The method blank will show contamination from laboratory reagents, from the air, and from other samples either on the instrument or during sample preparation. Phthalate contamination is common in the analysis of SVOCs. Common laboratory solvents such as methylene chloride and acetone, can be a real problem during purge-and-trap analysis of VOCs. The limits for the amount of a target analyte found in a blank are usually defined in the method or the QAP. For example, USEPA Method SW8000 defines the limit to be less than 5% of the regulatory limit for the compound or less than 5% of the analyte concentration found in the sample, whichever is greater (13).

15.17.3.4 Laboratory Control Samples (LCSs)

The LCS is a clean matrix that is fortified with the target analytes and carried through the analytical method. An aqueous LCS can be tap water, deionized

water, or deionized water that has common salts added to it to mimic natural waters. Baked sand or common topsoil are often used for the soil LCS. One LCS is analyzed per batch of 20 samples or daily. The LCS monitors method performance in the absence of matrix effects, which are common to many environmental samples. Recovery limits are established for each target analyte in the LCS. Typically failure to demonstrate these recoveries requires reanalysis of the entire batch of samples. Comparison of recoveries obtained from the matrix spike samples to the LCS can be used to show sample matrix effects. Sometimes the LCS is also called a laboratory fortified blank (LFB).

15.17.3.5 Matrix Spike Samples and Duplicates (MSs and MSDs)

Environmental reference samples that contain organic compounds are rare because of the instability of organic compounds in soil and water. NIST supplies several for PCBs (whale blubber and marine sediment) and PAHs (urban dust). Method accuracy and precision are measured using the matrix spike (MS) and the matrix spike duplicate (MSD). The target analytes are spiked into duplicate aliquots of the sample and carried through the analytical method. Of course, the unspiked or background sample must also be analyzed for this data to have any meaning. Spike recoveries are calculated for the MS and MSD after subtracting the concentration of the analyte in the unspiked sample. Clean samples present no problem because they can be spiked so that the analyte concentration lies within the calibration range. However, highly contaminated samples must be spiked at concentrations high enough to assess recovery. Ideally the sample should be spiked to produce an analyte concentration that is at least two times that of the unspiked sample. Unfortunately with the analysis of SVOCs the sample must be spiked prior to extraction and the analyst often has no knowledge of what that concentration should be. Recoveries of analytes for the MS and MSD are usually less than quantitative, and recoveries of less than 50% for problematic compounds are not uncommon. Precision is assessed by calculation of the relative percent difference (RPD) between the MS and the MSD. The RPD can be expressed as the absolute difference between the concentrations of the analyte found in the MS and MSD divided by the average concentration found in the two samples. Control limits are established for both the recovery and the RPD for each analyte. When any of recoveries from the MS and MSD are outside the quality control limits, the recoveries are compared to those of the LCS to determine whether the problem can be attributed to the sample matrix. If the LCS recoveries were in specification and the MS or MSD were out of the control limit, then the result can be attributed to sample matrix effects. If the recoveries of the LCS are out of specification, then the sample batch must be reanalyzed. An MS and MSD are prepared with each batch of 20 samples or daily if less than 20 samples are analyzed on a given day.

15.17.3.6 Quality Control Charts

Spiking recoveries for each analyte in a particular method are recorded and plotted. These data are used to create control charts and establish recovery limits

for target compounds. By plotting recoveries on each QC sample, trends or biases in the analytical method can be observed. The mean recovery is determined from the historical data. The 95% confidence interval, which is approximately ± 2 standard deviations around the mean, is calculated to establish upper and lower warning limits for recoveries. The control limits are defined by ±3 standard deviations around the mean and represent the 99% confidence level. If a recovery for an analyte is outside the upper or lower control limit, the data are said to be "out of spec" and must be investigated. Recoveries falling outside the warning limit but within the control limit are "in spec," but should serve as a caveat to the analyst of potential problems with the method.

15.17.3.7 Performance Evaluation Standards (PESs)

Many of the USEPA require the injection of a PES to assess the performance of the gas chromatograph and column before the analysis of samples can begin. The PES may be used to show the resolution between two closely eluting target compounds to demonstrate that the column has an adequate number of theoretical plates to perform the separation of the target analytes. Compounds that break down in the injection port or on the head of the analytical column, such as DDT and endrin (see Section 15.13.1 on organochlorine pesticides) are often included in the PES to ensure that the gas chromatograph is in good condition. The PES will often include polar compounds, such as pentachlorophenol or benzidine. The peak tailing factors of these compounds are measured to assess activity in the gas chromatographic system and must be less than a specified limit before analysis of samples can begin.

15.17.4 Method Detection Limits and the Limit of Quantitation

According to USEPA terminology, the method detection limit (MDL) of an analyte is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. Many of the USEPA methods and regulations list an MDL for the each analyte on the target compound list. On a regular basis laboratories are required to perform MDL studies to demonstrate that they can achieve this level. Typically a clean matrix (deionized water or clean sand) is spiked with the target analytes at a concentration that is estimated to be close to, but slightly above the method detection limit. A good way to estimate the MDL is to use a concentration value that will produce an analyte peak with a signal-to-noise ratio of 2–5. A minimum of seven replicate samples are spiked at this level with each of the target analytes and carried through the entire analytical procedure including extraction and cleanup steps. After calculating the standard deviation(s) from the measured concentrations of the replicates the MDL can be calculated as

$$\text{MDL} = s \times t(n-1, \alpha = 0.01) \tag{5.13}$$

where s is the standard deviation of the replicates and $t_{(n-1,\alpha=0.01)}$ = students t at the 99% confidence level with $n-1$, degrees of freedom and n is the number of replicates (for seven replicates, $t = 3.14$).

The limit of quantitation (LOQ) is defined as being 5 times the MDL and is considered the minimum value that can be accurately quantified. Sometimes the LOQ is also referred to as the reporting limit. In some reporting formats data that is above the MDL and less than the LOQ is labeled with a "J" to indicate that the analyte was detected and that the concentration is estimated, because it is below the LOQ.

15.18 THE FUTURE OF GAS CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS

Several trends seem to be emerging in the gas chromatographic analysis of environmental samples. One major tend is the push for faster and greener sample preparation procedures. A greener technique is less harmful to the environment and safer for analysts. A major theme has been to limit the use of solvents in sample preparation, especially halogenated solvents. The interest in supercritical-fluid extraction (SFE) has tapered because of its limitations in extracting a wide range analytes, its lack of robustness, and poor dependability in extracting large numbers of samples. Unless new discoveries are made in the use of supercritical fluids and the dependability of commercial instrumentation is improved, SFE will continue to slide. The number of applications of solid-phase microextraction (SPME) has exploded in the last several years. SPME appears to be the sample prep of the future for gas chromatography and will probably continue to blossom because of its simplicity, speed, and the fact that it is a green technique requiring no solvent. It has the potential to replace the classical techniques for the determination of VOCs and SVOCs in air, water, and soil, but SPME methods must obtain regulatory acceptance from the USEPA and state agencies. Significant steps have already been made in understanding and improving quantification using SPME, but continued efforts are needed and sensitivities comparable to or better than the classical methods must be shown. Strategies must be developed to determine long lists of classes of compounds much in the same way VOCs, SVOCs, and pesticides and PCBs are now determined by classical methods. Use of solid-phase extraction should also continue to grow because of the savings in solvent consumption over the classical methods. USEPA methods are continually being converted to include the use of SPE. The solid-phase disks seem to have an edge on the cartridges, because their large surface area facilitates the rapid extraction of aqueous samples. Pressurized fluid extraction and microwave assisted extraction are advantageous over the conventional methods because less solvent is consumed, they are less labor-intensive, and faster extraction times can be realized. These techniques should gain wider acceptance in the coming years (see Chapter 11).

In the next decade many gas chromatographic separations used in environmental analysis will be converted to fast GC (see Chapter 5). Fast GC is a trend that has emerged since the mid-1990s. Separations requiring 30–40 min can be completed in very short times ranging from seconds to several minutes.

Narrower-bore columns (50–100 μm i.d.), more efficient means of temperature programming, and pneumatic programming has brought about fast GC. Detectors will continue to become smaller, faster, and more sensitive in the coming decade. The use of the mass spectrometer will continue to flourish as the primary detector for the gas chromatograph in analyzing environmental samples. Increases in sensitivity and scanning speed may eventually make it the only detector that will be used. The time-of-flight mass spectrometer (TOFMS), which can perform several thousand scans per second, can meet the requirements of a detector for fast GC. Methods like USEPA 8260B and 8270C (GCMS for VOCs and SVOCs, respectively) can to be converted to fast GCMS methods employing the TOFMS and peak separation algorithms to obtain runtimes under 5 minutes.

Chemical compounds that are known as the "endocrine disrupters" have become a major environmental concern. Simply defined, an endocrine disrupter is a chemical that interferes with normal function of the endocrine system. The Endocrine Disrupter Screening Advisory Committee (EDSAC) formed by USEPA in 1996 published its final report in 1998 and recommended that approximately 87,000 chemicals be screened as possible endocrine disrupters. Because approximately 25,000 of these compounds have a molecular weight greater than 1000 daltons (μ), and it is unlikely these compounds will cross the cell membrane, testing on these compounds will be postponed. The remaining 62,000 compounds will be screened as possible endocrine disrupters using a two-tier screening process. The first screening will consist of in vitro and in vivo assays with fish, frogs, and rodents to quickly detect estrogen, androgen and thyroid disrupters. Chemicals that test positive in the first screening will be subjected to further animal studies using reproductive and toxicity testing. Chemicals that are potential endocrine disrupters include

- Akyl phenols and alkyl ethoxylates
- Bisphenol A
- Metals and organometallic compounds
- Dioxin
- Hormones and metabolites such as testosterone, estradiols, and progesterone
- Organochlorine pesticides and PCBs
- Phthalates
- Pharmaceutical and personal care products (PPCPs) such antibiotics, anti-inflammatory medicines, blood lipid regulators, pain medication, and fragrances (129,130)

As the enormous task of screening for endocrine disrupters progresses over the next decade, new substances will require environmental regulation and monitoring. Although not all of these chemicals may be amenable to gas chromatography, gas chromatographic methods will be developed and necessary to monitor for these compounds in the environment.

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CHAPTER SIXTEEN

Forensic Science Applications of Gas Chromatography

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PART 1 INTRODUCTION

16.1 INTRODUCTION

Chromatography has become an important tool in many fields, including forensic science, and is widely used because of the versatility, sensitivity, speed, and reliability of the technique. Gas chromatography (GC) in particular has been successfully applied to a number of specific and unique problems encountered in the crime laboratory. There is good reason for this success. Since the early 1980s chromatographic instrumentation has become affordable to the routine crime laboratory and GC has been successfully interfaced to other techniques, specifically, mass spectrometry and infrared spectroscopy, allowing these sophisticated techniques to be applied to unique problems within the crime laboratory. In addition, the basic principles and theory of GC are readily understood, and the instrumentation has become reliable, simple enough for the novice to operate, and easily automated.

In this chapter gas chromatographic applications to problem samples, routine and nonroutine, encountered in the crime laboratory are discussed. The material is covered in sufficient detail to enable the reader to understand each specific application and to render an appreciation for the type of work in which GC is applied in forensic science.

16.1.1 Definition and Scope of Forensic Science

Forensic science can be broadly defined as the application of science to law. It is more commonly applied to those laws (criminal and civil) that are enforced by police agencies in the criminal justice system. Specifically, it is the application of the principles of chemistry and related sciences to the examination of physical evidence collected at the scene of a crime, and the interpretation of the results of that examination in a court of law by an expert.

In the general field of forensic science many disciplines of science have been applied. Some do not use GC in their particular application and are beyond the scope of this chapter. For example, these would include such areas as forensic archeology, forensic engineering, forensic anthropology, fingerprint examination, forensic odontology, forensic psychiatry, and many more.

There are, however, a number of different types of forensic science that do apply GC to solve some unique problems. These are the areas of forensic science from which the examples are taken and that are addressed in this chapter.

Most have to do with the area of drugs of abuse and toxicological analyses, including blood alcohol analysis. There are also some very unique applications in criminalistics, such as analysis of debris from fire scenes for accelerants, explosive analysis, and the examination of trace evidence, such as paints, fibers, and other polymers. Gas chromatographic applications within these specific areas are detailed in the chapter.

16.1.2 Functions of the Forensic Scientist

The forensic scientist's responsibility in all areas of forensic science is to analyze the evidential material with the best analytical techniques available and report the findings to the requesting authority. It is also the responsibility of the scientist to testify in court about the results of the tests performed, the scientific techniques used, and, if applicable, the meaning and interpretation of the data presented. The scientist must be able to articulate and explain to laypeople, such as lawyers, police officers, and general citizens with no scientific background, the technical nature of the work in terms that nontechnically trained people can understand. The court testimony of a forensic scientist may or may not be accepted. His or her familiarity with the topic may be questioned, and any failure to respond effectively creates uncertainty in the minds of the judge and jury. The forensic scientist can perform the most sophisticated analytical chemistry in the laboratory, but if the results are not communicated properly, the value of the analysis may be lost.

During testimony, the scientist must also be aware of the demands of the courts. The procedures used in the laboratory must not only rest on a firm scientific foundation but must also satisfy the criteria of admissibility. The primary rules for scientific and expert evidence are governed by federal and state statutes, the Federal Rules of Evidence, and case law (1–3). GC is a technique that is generally accepted by the scientific community as a reliable procedure in the analysis of physical evidence and has met all the requirements imposed by the judicial system when properly applied.

Forensic scientists are also called on to train law enforcement personnel regarding the capabilities of the laboratory. Prosecutors and investigators must understand the capability of the forensic laboratory and the value of the analyses that may be performed there. They need not know the theory and operation of the gas chromatograph, but a general knowledge of how evidence is examined can be helpful for the investigator to properly recognize and collect evidence.

16.2 PHYSICAL EVIDENCE

16.2.1 Types of Evidence

Physical evidence encompasses any and all objects that can establish that a crime has been committed or can provide a link between a crime and its victim or a crime and its perpetrator. The ultimate goal in examining physical evidence is to

TABLE 16.1 Common Types of Physical Evidence

Blood[a]	Fibers[a]	Organs[a]
Semen	Fingerprints	Other physiological fluids[a]
Saliva[a]	Firearms and ammunition	Petroleum products[a]
Documents	Glass	Powder residues
Drugs[a]	Hair	Serial numbers
Explosives[a]	Impressions	Solids and minerals
Toolmarks	Polymers[a]	Wood and vegetation
Paint[a]	Soil	

[a]Commonly analyzed by GC.

help determine or reconstruct the events of the crime and if possible the order of events.

Physical evidence can be any type of material, small or large, that can help in linking the victim(s) or suspect(s) to the scene(s) or to each other. Table 16.1 lists some common types of physical evidence. The evidence with asterisks is commonly analyzed by gas chromatographic techniques and will be discussed in more detail later in the chapter.

16.2.2 Identification versus Comparison

In the analysis of physical evidence the significance placed on that evidence depends on how narrowly the evidence can be related to the source. This is where forensic science differs from most other types of science. Generally, other sciences are satisfied when an object can be placed into a specific class of the discipline. Criminalistics, or more generally, forensic science, strives to relate the object to a particular source. This can be accomplished in one of two ways.

The evidence may need an identification of the particular substance, or it may need to be compared to a standard or comparison sample. If identification is needed, then a determination of the physical or chemical identity of a substance with as near absolute certainty as existing analytical techniques will permit must be performed. For example, the identification of a particular drug, an accelerant, or a type of explosive may be needed. GC can play a key role in this identification, especially when it is interfaced with highly specific detectors such as mass spectrometers or infrared spectrophotometers.

When a comparison analysis is needed, the suspect evidence is subjected to the same set of tests as a comparison piece of evidence for the ultimate purpose of determining whether they have a common origin. GC is also used effectively in this type of analysis. For example, paint samples are routinely compared in the forensic laboratory by pyrolysis GC (PGC) to determine the source of origin.

16.2.3 Class versus Individual Characteristics

When a comparison analysis is undertaken in the forensic laboratory, a two-step process must be performed: (1) the best possible distinguishing properties must

be selected for comparison of both the suspect and control evidence specimens and (2) more important, it must be decided whether a conclusion can be drawn from the data as to the origin of the suspect evidence and control evidence. In other words, do both pieces of evidence come from the same source?

When considering the conclusion as to the origin of physical evidence, the significance of the data and the power of differentiation both contribute to the value of the physical evidence. For the most part, physical evidence falls into two classifications: physical evidence with class characteristics only and physical evidence with individual identifying characteristics. When evidence is classified as having class characteristics only, such evidence, no matter how thoroughly examined, can only be placed into a class. A definite conclusion as to common origin can never be made since there is a possibility of more than one source of the evidence. An example of this type of evidence would be a single-layered paint chip or a footprint from a new pair of sneakers.

When evidence is classified as having individual identifying characteristics, this evidence can be definitely attributed to a person or source. The classic example of this type of evidence would be a set of fingerprints, since no two people have the same set of prints. GC can also be valuable in obtaining individual identifying characteristics, for example, in cases such as the establishment of the origin of two drug specimens.

Of course, it is always desirable to have evidence that can be positively individualized, but the value of class evidence only should not be minimized. Class evidence can be valuable in an investigation where there is a preponderance of such evidence.

For further reading about forensic science and the analysis of physical evidence, the reader is directed to general texts on forensic science (4–7).

PART 2 DRUG ANALYSIS BY GAS CHROMATOGRAPHY

16.3 CONSIDERATIONS IN FORENSIC DRUG ANALYSIS WITH GAS CHROMATOGRAPHY

16.3.1 Introduction to the Analysis of Drugs of Abuse

Drug abuse has become a major problem for the United States and many other countries around the world. The analysis of drugs of abuse now accounts for a major proportion of the workload of state and local forensic science laboratories. The most commonly encountered drugs of abuse at the present time are cannabis (marijuana), cocaine (crack), heroin, phencyclidine (PCP), lysergic acid diethylamide (LSD), amphetamines, including 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA), and other designer drugs.

The use of GC in the analysis of drugs of abuse is well established (8–10). A large number of methods with varying conditions are used for different drugs. The most common detectors employed for the gas chromatographic analysis of

drugs of abuse are the flame ionization detector (FID), the mass-selective detector (MSD), and mass spectrometers. For nitrogen-containing drugs, alkali flame ionization detectors (AFIDs) or flame-photometric detectors are used in detection for added sensitivity and selectivity. In addition, infrared spectrophotometric detectors (IRD) have been used for more volatile drugs and electron-capture detectors (ECD) have been used after derivatization of the drug for added sensitivity and selectivity.

16.3.2 Controlled Dangerous Substance (CDS) Laws and Schedules

There are several ways to classify drugs of abuse. The most relevant classification to forensic science is the legal classification whereby the federal and state codes classify drugs of abuse as "controlled substances" by schedule. For the purposes of this chapter the discussion of the application of GC to drugs of abuse is limited to controlled substances. For the application of GC to other drugs, the reader is referred to Chapter 14 on clinical applications of GC.

For practical law enforcement purposes the legal community has outlined the drug classifications and definitions in the drug laws. These laws are of particular interest to the forensic scientist, since they may impose certain requirements as to the analytical protocol for drug analysis. For example, the severity of a penalty associated with the manufacture, distribution, possession, and use of a drug may depend on the identification of a particular active compound, the weight of a drug, or the concentration of the drug. In these particular cases, the appropriate analytical approach must be performed and the drug analysis report must contain the pertinent information.

The Controlled Substance Act regulates the handling of drugs of abuse (11). There are five schedules, or lists, that classify drugs according to medical use and degree of abuse:

- Schedule I drugs have no accepted medical use in the United States but a high rate of abuse and/or lack accepted safety for use in treatment under medical supervision. Drugs controlled under this schedule include heroin, marijuana, methaqualone, gamma-hydroxybutyrate (GHB), 3,4-methylenedioxymethamphetamine (MDMA), and LSD.
- Schedule II drugs have an accepted medical use in the United States and a high rate of abuse, with either severe psychological or physical dependence potential. These drugs include morphine, codeine, cocaine, amphetamine, and most barbiturate preparations containing amobarbital, secobarbital, and pentobarbital.
- Schedule III drugs have an accepted medical use, but a lower potential for abuse than do Schedules I or II, and have a potential for low or moderate physical dependency or high psychological dependency. Examples are all barbiturate preparations (except phenobarbital) not covered under Schedule II, some codeine preparations, and steroid preparations, such as testosterone and its esters.

- Schedule IV drugs have an accepted medical use and generally have a low potential for abuse relative to Schedule III. Drugs controlled under Schedule IV are generally the long-acting barbiturates, hypnotics, and minor tranquilizers, such as meprobamate, phenobarbital, diazepam, and dextropropoxyphene.
- Schedule V drugs have medical use, have low abuse potential, and have less potential for producing dependency than do Schedule IV drugs. These drugs may be any of the drugs in the schedules listed above, usually in solution, at low concentration of controlled substance, which also may contain noncontrolled ingredients in sufficient quantities to effect qualities other than those possessed by the controlled substance alone; cough syrup is an example.

Under the Controlled Substance Act, a drug must be specifically classified in one of the five schedules if its use is to be considered illegal. This requirement has given rise to the existence of the "designer drugs" as a means of circumventing this law. Designer drugs are substances that are chemically related to that of a controlled drug in Schedule I or II and are pharmacologically very potent. In response to the legal problems with scheduling designer drugs, the "Controlled Substance Act" has been updated to include a provision stipulating an offense involving a controlled substance analog, a chemical substance substantially similar in chemical structure to a controlled substance.

More recent changes in the Controlled Substances Act also regulate the manufacture and distribution of precursors, the chemical compounds used by clandestine drug laboratories to synthesize drugs of abuse.

Controlled drugs can be procured only from licensed sources, and accurate records of inventory must be kept as well as the amount of drug used. Dilute standard solutions can be purchased from commercial sources, in limited quantities, usually without difficulty or license.

16.3.3 Types of Physical Evidence: Sample Preparation

A classification scheme for drugs of abuse that is of more practical interest is classification by the form(s) in which the drug substance is most often found when submitted as evidence to the laboratory.

Controlled substances are found in three major forms. The first type is plant or vegetative material. Marijuana, peyote, khat (*Catha edulis*), and mushrooms that contain psilocybin fall into this category. Substances in this form usually require some botanical examination as well as chemical analyses. To prepare samples for analysis by GC, a specialized extraction procedure is normally required to separate the naturally occurring compounds from the drug to be chromatographed.

Another form of drug submissions is labeled tablets and capsules. For the most part, these are legitimately manufactured and generally bear identification marks that can be searched in the *Physician's Desk Reference* (PDR) for the identification of its contents.

The third form in which drugs of abuse are submitted to the laboratory is in powder form. This is usually classified as a general unknown and grouped with other types of submissions that are in the liquid form. Drugs of abuse of this type vary, such as cocaine HCl, cocaine free base (crack), heroin, PCP, and methamphetamine. This form of drug is frequently adulterated, which has important implications in sample preparation and analytical methodology. Other unmarked tablets and capsules, including clandestinely manufactured LSD, as well as steroid preparations, can be classified into this category.

Since illicit drugs are rarely encountered in their pure form, it is usually necessary to extract the drug of interest from any interfering compounds or adulterants before the gas chromatographic analysis. While dry extractions can be performed, one-step liquid–liquid extractions are usually more effective. The analyte drug is extracted from an acidic or basic solution into an organic solvent such as methylene chloride or ether. Drugs of abuse may be classified as either acidic, basic, or neutral, depending on their pK_a. This classification is extremely useful to the forensic drug examiner in deciding on the appropriate methodology. Examples of basic drugs are heroin, cocaine, PCP, and amphetamines. Barbiturates are examples of acidic drugs.

Solid-phase extraction (SPE) may be used to separate drugs from interfering materials; however, this procedure is seldom used for bulk formulations on a routine basis but is generally used for toxicological analyses. Liquid–liquid extraction schemes can also require several steps, including extraction, filtration, centrifugation, and evaporation stages, which increase the analysis time of a single sample. However, when the analyst has completely prepared the sample for analysis, the sample is relatively clean, with the drug remaining in a few microliters ($\sim$50+) for analysis by GC.

Solid-phase microextraction (SPME) is a recent sample preparation technique for trace analysis by GC (12). It is a simple, solvent-free method that uses a polar or nonpolar coated fused-silica fiber to directly extract analytes from various matrices (usually aqueous). It can be used in a headspace mode as well. After the fiber is removed from the sample, it is transferred to the heated inlet of a chromatographic system and the analytes are thermally desorbed for analysis. The technique works well for the analysis of trace analytes in water or urine. It has been applied in the field of forensic science in the analysis of fire debris, explosives, and drugs in biological fluids (13–15).

Preparing the sample for GC is only one step in the examination of controlled dangerous substances. Several steps must be taken in the scheme of analysis of an unknown drug submission. Table 16.2 lists the steps in a general scheme of analysis for a typical unknown drug submission.

There is, of course, no one scheme of analysis for all drugs of abuse. Different laboratories may use different schemes for the same drugs. The methodology depends on a number of different factors, which include but are not limited to the particular drug, the laws that govern the particular locale or state, the instruments available in the laboratory, the number of cases submitted annually, the number of personnel available to analyze the case, and the training and

TABLE 16.2 General Scheme of Analysis of Drugs of Abuse

Preliminary visual examination of all specimens
Weights of all exhibits; volume (if liquid is present)
Selection of representative samples
Microscopic examination (if vegetation)
Screening tests (usually spot tests, may include UV spectrophotometry, thin-layer chromatography, HPLC, and/or gas chromatography)
Microcrystalline tests (optional)
Extraction
Confirmatory test (GCMS, HPLCMS, IR, or Raman spectrophotometry)
Quantitative analysis (if needed)

background of the scientist. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has recommended minimum standards for forensic drug identification, which includes the use of gas chromatography (16).

For further reading on the analysis of drugs of abuse the reader is referred to an excellent chapter written by Siegel (17) and a comprehensive book on the subject written by Gough (9). For more detailed information on analytical data, References 18–27 are recommended.

16.4 QUALITATIVE ANALYSIS OF DRUGS OF ABUSE

Controlled drugs of abuse can generally be classified into five broad areas based on the drugs effects. Table 16.3 lists some examples of commonly abused drugs according to these five classes. GC is routinely used in the forensic laboratory to separate drugs within each class. The following discussion illustrates some examples of the analysis of drugs of abuse by GC.

16.4.1 Narcotics

The class of narcotic drugs encompasses the opium-derived drugs of morphine, heroin, and codeine (Figure 16.1) as well as other narcotics, such as meperidine, hydromorphone, hydrocodone, and the fentanyl compounds. Because these are highly polar compounds and often require high temperatures for elution, GC is difficult and often demands derivatization. Morphine, for example, because of its amphoteric nature, is not only difficult to extract but must be derivatized to obtain good quantitative data.

Heroin (Figure 16.1a) is the most widely abused semisynthetic opiate. It was first synthesized in 1874 by acetylation of morphine (Figure 16.1b). Heroin is usually seen as a white crystalline powder and is mixed with diluents in illicit samples, such as sugar, quinine, caffeine, and even strychnine. Therefore it is necessary for chromatographic systems to resolve heroin and its commonly encountered diluents. In street samples heroin may comprise as low as 2% by weight of the total sample, which causes difficulty with separation when it is

TABLE 16.3 Classification of Some Commonly Abused Drugs

Class	Drug
Narcotic	Morphine
	Heroin
	Codeine
	Methadone
	Fentanyl
Stimulant	Amphetamines
	Cocaine (crack)
Depressant	Barbiturates (both short- and long-acting)
	Ethanol
	Gamma-hydroxybutyrate (GHB)
	Meprobamate
	Diazepam (Valium)
	Chlordiazepoxide (Librium)
Hallucinogens	Marijuana
	PCP
	LSD
	3,4-Methylenedioxyamphetamine (MDA)
	3,4-Methylenedioxymethamphetamine (MDMA) (Ecstasy)
Steroids	Testosterone
	Stanzolone
	Testosterone esters

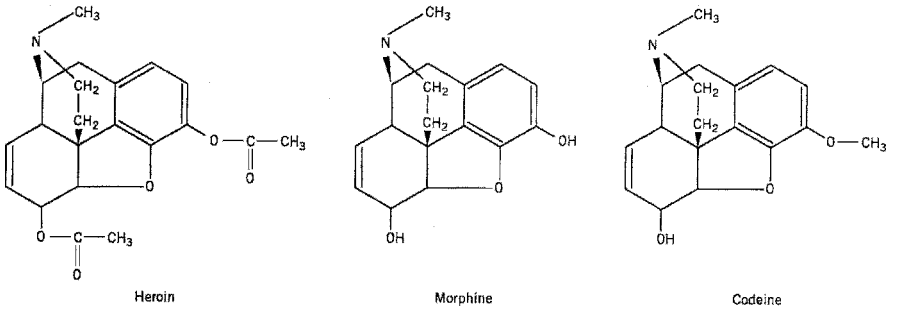


FIGURE 16.1 Structures of (a) heroin, (b) morphine, and (c) codeine.

present in very complex mixtures. Heroin at its point of origin is not very pure, since it usually contains some acetylcodeine, morphine, and monoacetylmorphine. Analysis of illicit heroin and its impurities can be performed by GCMS. A method has been developed for the simultaneous determination of heroin along with some of the commonly occurring adulterants utilizing simple dissolution of the sample along with an internal standard followed by capillary GC on a nonpolar methylsilicone column attached to a FID (28).

Heroin historically has been introduced into the body by intravenous syringe; however, since the early 1990s, heroin has shown signs of increasing purity to

30–40% or higher, indicating a user shift to nasal introduction ("snorting") (29). Wyatt and Grady (30) have reviewed the physical properties, synthesis, stability, metabolism, and analysis of heroin. A review of laboratory methods for the analysis of opiates and diluents in illicit drugs has been reported (31).

16.4.2 Stimulants

The most commonly encountered compounds in this class are the phenethylamines. These include amphetamine, methamphetamine, phentermine, and many other structurally related compounds that are controlled according to the Controlled Substance Act. Several factors make this class of drugs the most difficult to analyze. Because the phenethylamines are the most commonly clandestinely manufactured class of drug, the variety of closely related structural compounds can make the analysis a complicated process, requiring the GC to have high resolution, selective liquid phases, and good sensitivity. In addition, other structurally related compounds that are not controlled by law but are legitimately made are often found in these samples. These include ephedrine, phenylpropanolamine, and caffeine.

Amphetamine salts produce poor results during gas chromatographic analyses. The compounds must be prepared by extracting a basic solution of amphetamines into an organic solvent such as methylene chloride. This will produce a sample of free-base amphetamine which can be chromatographed easily. The use of methanol or ethanol as the injection solvent for gas chromatographic analysis of amphetamines is not recommended. The primary amines, such as amphetamine, MDA, and phenethylamine, yield imines on injection of methanol or ethanol solutions (32). For this reason and because free-base amphetamines generally yield nondiscriminating mass spectra, several different derivatization techniques have been employed for the separation of these compounds. The formation of Schiff bases or conversion to amides are procedures that have been used, but halogenated derivatives account for many techniques that have sought better selectivity and sensitivity with the use of an electron capture detector (33).

Trichloracetyl and 4-carbethoxyhexafluorobutyryl chloride derivatives have been used to increase the molecular weight of the amphetamines. These particular derivatives consequently lengthen the elution times and help separate these compounds from potential interference (34,35). *N*-Monotrifluoroacetylated (TFA) derivatives of amphetamine analogues have been prepared by on-column derivatization with *N*-methylbis(trifluoroacetamide) (MBTFA) (36). A separation on a 12-m methylsilicone capillary column of four phenethylamines with closely related structures is shown in Figure 16.2 using this procedure.

Chiral separation of amphetamines has also been performed. The enantiomeric composition of amphetamine samples can provide information about the synthesis and origin of drugs. For example, Liu et al. (37) used *N*-trifluoroacetyl-L-prolychloride (TPC) to separate isomers of methamphetamine on two different columns.

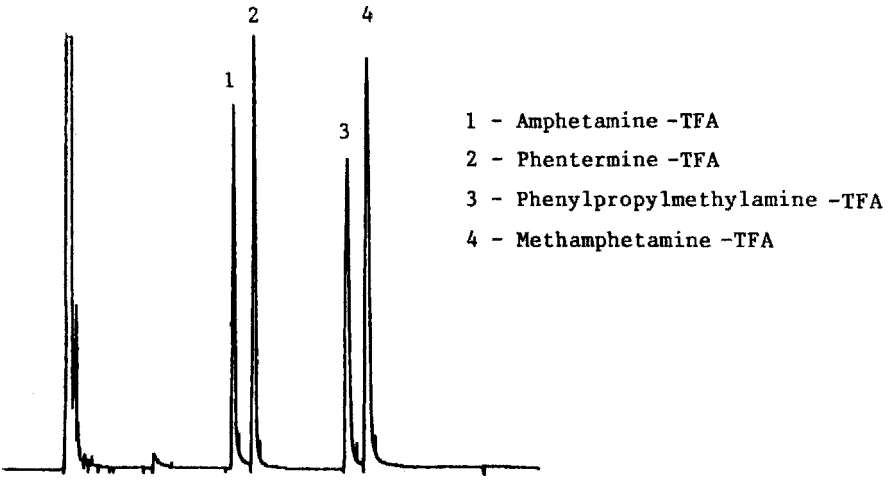
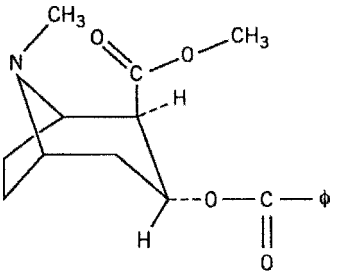


FIGURE 16.2 Separation of the TFA derivatives of amphetamine, methamphetamine, phentermine, and phenylpropylmethylamine using on-column derivatization with MBTFA. GCFID conditions: 12-m HP-1 × 0.20-mm i.d. × 0.33-μm film; 120–180°C at 30°C/min; split ratio = 20/1. (Courtesy of New Jersey State Police.)

16.4.3 Cocaine

Cocaine, a benzoic acid ester of ecgonine (Figure 16.3), is a naturally occurring alkaloid from the plant *Erythroxylon coca,* grown in South America. Cocaine is a nervous system stimulant and local anesthetic drug and is one of the most widely abused drugs in the United States. The most common route of administration is intranasal (IN) by insufflation or snorting. A more popular form of cocaine is the free-base form, known as "crack". Crack is made by alkalinizing the salt, usually with bicarbonate, and extracting into nonpolar solvents. The drug in this form can be smoked by the abuser, which produces a quicker and more intense euphoria.



l-Cocaine

FIGURE 16.3 Structure of *l*-cocaine.

Illicit cocaine can be cut with a number of different diluents, such as tetracaine, lidocaine, benzocaine, and procaine, to mimic the numbing sensation of cocaine when taste-tested. Other additives are mixed with cocaine to add bulk, such as sodium bicarbonate, starch, talcum powder, boric acid, and sugars.

Cocaine can be separated from the other caine diluents on a 12-m × 0.20-mm-i.d., 0.33-μm film thickness capillary column using an isothermal temperature of 220°C in a very short runtime (>5 min). Figure 16.4 shows the separation of a mixture. Capillary column methods with conditions similar to those shown in Figure 16.4 have replaced the packed column methods used in previous years simply because they provide the best resolution in the shortest analysis time. Capillary GC coupled to a selective detector such as an NPD or a mass spectrometer has become the method of choice for analyzing illicit cocaine samples.

Coca paste and several fractions from smoking products have been analyzed by GCFID and GCMS (38). The inhalation efficiency and pyrolysis products of cocaine by the pyrolysis of crack and cocaine hydrochloride have been studied by GC and GCMS (39). GCMS has also been used to study the injection-port-produced artifacts from cocaine (crack) exhibits (40). The detection of cocaine on various denominations of United States currency has been reported using solid-phase extraction and GCMS (41).

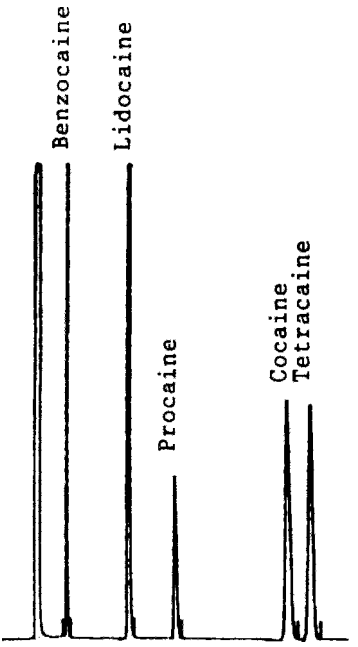


FIGURE 16.4 Separation of benzocaine, lidocaine, procaine, cocaine, and tetracaine. GCFID conditions: 12-m HP-1 × 0.2-mm-i.d. × 0.33-μm film; oven = 220°C, injection = 250°C, detector = 300°C; split ratio = 20/1. (Courtesy of New Jersey State Police.)

16.4.4 Barbiturates

A variety of gas chromatographic methods have been reported for the analysis of barbiturates. The first chromatographic separation of barbiturates was described in the 1960s. Since that time most of the work performed has been with derivatization techniques because free barbituric acids exhibit considerable adsorption and peak tailing. Jain and Cravey (42) have reviewed the literature up to 1974, and Pillai and Dilli (43) have reviewed the analysis of barbiturates by GC up to 1980. With the advent of modern fused-silica capillary columns the need for derivatization has lessened; however, some barbiturates give very similar mass spectra and diligent use of retention times is needed for additional identification. For this reason most analysts still use derivatization. Many reagents have been used, including BSTFA for silanization, trimethylanilinium (TMAH) for methylated compounds, and pentaflourobenzyl bromide (PFBB) for halogenation. Alkyl derivatives seem to enjoy the greatest success, especially when trimethylphenylammonium hydroxide (TMPAH) is used.

Sample preparation requires a simple acid/organic solvent extraction or a direct extraction into ethanol being dried down and then subsequently injected into the chromatographic column. Methyl- or phenylmethylsilicone columns have been used with most success.

16.4.5 Benzodiazepines

Benzodiazepine drugs are used as muscle relaxants and were introduced in the 1960s. These drugs have characteristically shown long retention times, but with better stationary phases and the use of stable fused-silica capillary columns, this has been improved.

Some compounds in this particular class of drugs tend to break down during gas chromatographic analysis. For example, chlordiazepoxide (Librium) breaks down to *N*-desmethyldiazepam. Clorazepate presents several problems in identification. In addition to rapid acid decarboxylation to *N*-desmethyldiazepam, extracts of the pharmaceutical forms of clorazepate contain substances that interfere with isolation of intact and unaltered clorazepate. The analyst should be cautioned about this particular problem. Many procedures use HPLC for the analysis of benzodiazipines for these reasons. However, procedures involving solvent extractions, GC, and detection via FID, NPD, or MS (MSD) give good detection limits (nanograms) for easy identification.

Historically, OV-1 and SE-30 phases have been used for packed columns, and retention indices have been listed for this class of compounds for these phases (44). Plotczyk and Larson (45) have also studied the behavior of this class of drugs on 5% phenylmethylsilicone (DB-5, SE-52) fused-silica columns.

16.4.6 Cannabinoids

Cannabis usually comes in three forms: (1) cannabis (marijuana), (2) cannabis resin (hashish), and (3) extracts of cannabis resin (hashish oil). Most laboratories use a color test (modified Duquenois–Levine), a morphological examination

using a microscope, and thin-layer chromatography (TLC) to identify cannabis. Consequently, GC has not been widely used for the forensic identification of Cannabis samples.

Novotny et al. (46) were the first to indicate that there might be a correlation between the capillary gas chromatographic profiles and the country of origin. Brenneisen and El Sohly (47) later were able to differentiate samples of different origins using high-resolution capillary columns. Programmed temperature gas chromatographic analysis with high-resolution capillary columns can give information about batch origin, geographic origin, or identification of cannabinoids.

GCMS analyses of cannabis have identified over 400 compounds in the plant with 61 of these being cannabinoids (48). The major cannabinoids—cannabidiol (CBD), tetrahydrocannabinol (THC), and cannabinol (CBN)—can be readily separated on a 12-m methylsilicone column and identified using a mass spectrometer. Sample preparation simply consists of an organic solvent wash with petroleum ether.

One of the major applications of GC in the forensic analysis of cannabis has been the determination of total THC content (including decarboxylated THC acid), which is used to determine the quality of the cannabis product. Although gas chromatographic analysis using different temperature programs can give information about the geographic origin, little success has been achieved in this area by comparing cannabinoids due to the decarboxylation on injection of the sample. Although a wide range of stationary phases and supports has been studied, most recent work has been exclusively conducted on fused-silica capillary columns. For example, the cannabinoid acid pattern of plant preparations from *Cannabis sativa* (hashish, marijuana) has been determined by a hexane extraction and by analysis of their methyl-TMS derivatives with high-resolution GC and GCMS (49).

16.4.7 Hallucinogens

The most commonly abused hallucinogens are phencyclidine (PCP), lysergic acid diethylamide (LSD), psilocybin, mescaline, and 3,4-methylenedioxyamphetamine (MDA) and its analogs 3,4-methylenedioxymethamphetamine (MDMA) and its analogs, and 3,4-methylenedioxyethylamphetamine (MDEA). These compounds vary in structure and require different chromatographic conditions for analysis. Psilocybin, for example, requires derivatization before chromatographic analysis and many extractions cleave the phosphoryl group from psilocybin converting it to psilocin. HPLC is an alternative technique for separation of this particular drug from other impurities.

LSD has isomers that complicate its chromatography. LSD, iso-LSD, and LAMPA, the methyl-propyl analog of LSD, all have molecular weights of 323 amu and similar mass spectra. The resolution of LSD and LAMPA is difficult to achieve since LSD suffers thermal degradation. The separation of LSD and LAMPA is best achieved with a short, nonpolar fused-silica capillary column. Nichols et al. (50) used a 5-m SE-30 fused-silica capillary column and hydrogen

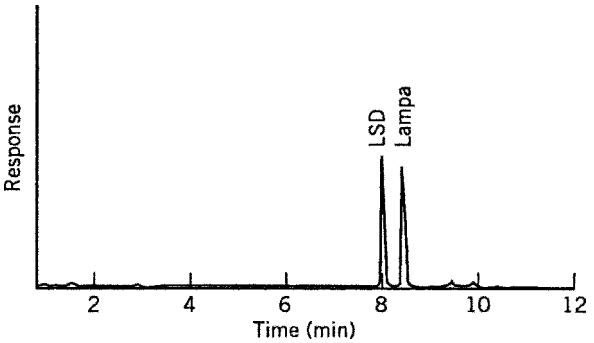


FIGURE 16.5 Separation of LSD and LAMPA. GCMSD conditions: 12-m HP-1 × 0.2-mm-i.d. × 0.33-μm film; 240–280°C at 10°C/min to 300°C at 5°C/min, injection = 270°C, split ratio = 20/1. (Courtesy of New Jersey State Police.)

as the carrier gas to obtain baseline resolution of these two compounds within 3 min. Figure 16.5 shows the baseline separation of LSD and LAMPA on a 12-m HP-1 fused-silica capillary column. It is essential that confirmation of the compounds in this class be accomplished by mass spectrometry because of the closely related isomers.

Phencyclidine (PCP), first sold as an animal tranquilizer, has been abused for years. PCP is submitted to the laboratory as either a powder or a liquid or may be found on vegetation such as marijuana or cigarettes in either a crystalline form or sprayed on as a liquid. The sole source of PCP is from clandestine laboratories, which means that the illicit samples are almost never pure but are mixed with precursors and byproducts of the synthesis. The contamination of illicit PCP by the carbonitrile precursor piperidinocyclohexanecarbonitrile (PCC) is a likely possibility. In addition, several other PCP derivatives have been identified in street samples. Table 16.4 lists some homologs and analogs to PCP that can be found in illicit samples. Any or all of these compounds could be misidentified for PCP if the proper analytical conditions are not controlled. For the reasons above, high resolution is required for separation and identification.

Separation of PCP analogs is accomplished quite easily, with medium to nonpolar phases offering the best chromatography (51). PCP has often been observed

TABLE 16.4 Homologs and Analogs of Phencyclidine

Abbreviation	Drug	Analog/Homolog
PCP	1-(1-Phenylcyclohexyl)piperidine	Phencyclidine
TCP	1-[1-(2-Thienyl)cyclohexyl]piperidine	Analog
PHP	1-(1-Phenylcyclohexyl)pyrrolidine	Homolog
PPP	1-(1-Phenylcyclopentyl)piperidine	Homolog
PCM	1-(1-Phenylcyclohexyl)morpholine	Analog
TCM	1-[1-(2-Thienyl)cyclohexyl]morpholine	Analog

to decompose to 1-phenylcyclohexane (PCH) on gas chromatographic analysis due to thermal degradation and the presence of acidic sites (52). This can be eliminated by the use of fused-silica capillary columns and relatively low temperatures.

16.4.8 Anabolic Steroids

The federal Anabolic Steroids Control Act of 1990 classified all compounds of anabolic activity as Schedule III controlled dangerous substances effective February 27, 1991 (53). Most state legislatures signed similar laws into effect shortly after this date, regulating the manufacture, distribution, or dispensing of anabolic steroids. The potential for abuse of anabolic steroids or androgens lies in the fact that they are responsible for many of the characteristics associated with male development. Athletes have used anabolic steroids since the 1950s to enhance athletic performance. In 1974, the class of anabolic steroids was added to the list of barred substances by the International Olympic Committee (IOC). Today, it is not just the competitive athletes who are abusing steroids but also those who want to improve their own physical appearance.

All anabolic steroids contain a five-membered cyclopentane ring fused to a fully reduced phenanthene ring system, similar to testosterone (Figure 16.6). Substitutions at the 3, 5, 9, and 17 positions give a variety of steroids with different properties. The variety and number of structurally related steroids makes the analysis of these compounds extremely complex for both the clinical samples and bulk formulations. Anabolic steroids can be analyzed by a variety of chromatographic techniques, including GC, HPLC, TLC, and supercritical-fluid chromatography (SFC). However, the most reliable technique for specificity and sensitivity is GCMS. Many GCMS methods exist for the determination of steroids. Most of these procedures were designed to detect steroids in biological fluids. Because metabolites and endogenous materials are present in biological samples and steroids are present at very low levels, sensitivity and specificity are absolute necessities for these procedures. To meet these requirements, derivatization procedures are universally used to screen, detect, and identify anabolic steroids by GCMS. Many of the derivatization procedures are discussed in the *Handbook of*

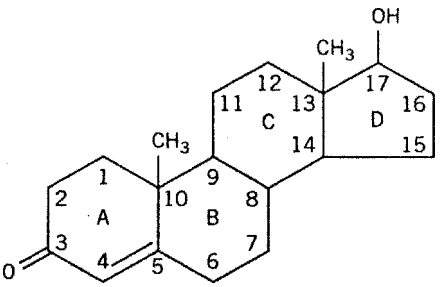


FIGURE 16.6 Structure of testosterone.

Chromatography: Steroids (54). The methyloxime–trimethylsilyl ether derivative has become the method of choice for derivatizing steroids. The success of this derivatization procedure is due to the ease by which both carbonyl and hydroxyl groups can be protected.

In contrast to clinical samples, bulk formulations provide more than an adequate amount of sample for analysis. Derivatization procedures are really unnecessary for unequivocal identification if adequate resolution can be achieved. Figure 16.7 shows the analysis of a mixture of 19 anabolic steroids on a 30-m × 0.25-mm-i.d. Rtx-5 (0.1-μm film thickness) fused-silica capillary column. Table 16.5 lists the corresponding compounds in this mixture. An analysis time of less than 18 min can be achieved using this column. Film thicknesses greater than 0.1 μm cause longer retention times and a corresponding deterioration in peak shape (55).

Retention time is also strongly influenced by the choice of stationary phase. Several factors must be taken into account when selecting a column for separating androgens. Retention time and resolution will be affected by the choice of column length, stationary film thickness and polarity, and the temperature program rate. The programmed temperature optimization of a mixture of anabolic

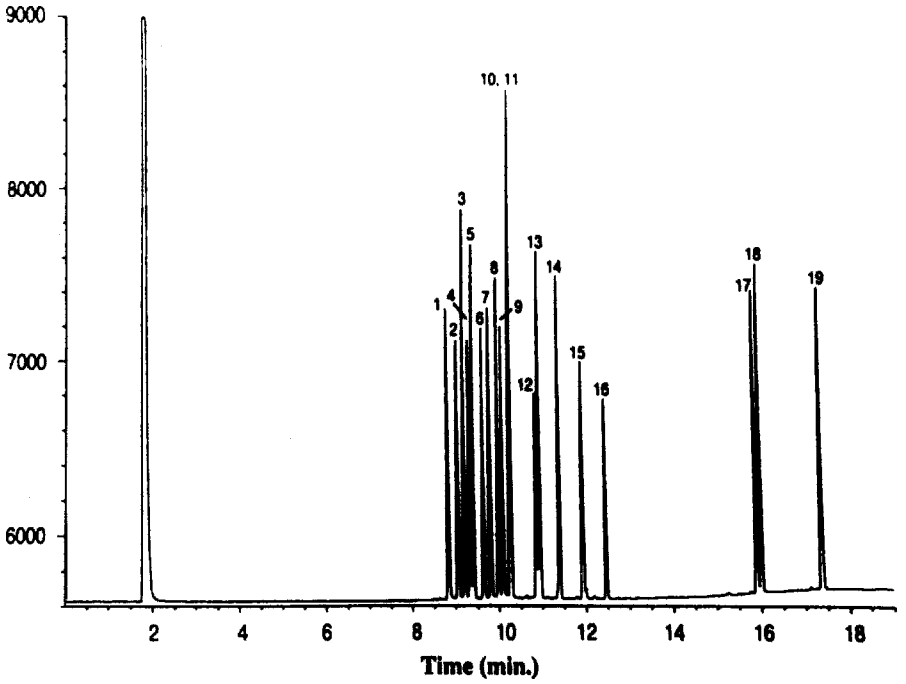


FIGURE 16.7 Separation of anabolic steroids. GCFID conditions: 30-m Rtx −5 × 0.25-mm-i.d. × 0.10-μm film; 180–340°C at 10°C/min (hold for 3 min); injection = 280°C, detector = 340°C; split ratio = 50/1, 1-μL split injection, concentration = 1000 ng/μL. (Reprinted with permission from Reference 55.)

TABLE 16.5 Anabolic Steroids in the Mixture of the Chromatogram from Figure 16.7

Peak Number	Anabolic Steroid
1	5-Androstene-3β,17β-diol
2	17α-Methyl-5-androstene-3β,17β-diol
3	5α-Androstan-17β-ol-3-one
4	19-Nortestosterone
5	17α-Methylandrostan-17β-ol-3-one
6	Mesterolone
7	Testosterone
8	17α-Methyltestosterone
9	1-Dehydrotestosterone
10	1-Dehydro-17α-methyltestosterone
11	Bolasterone
12	Oxymethalone
13	19-Nortestosterone-17-propionate
14	Testosterone propionate
15	Fluoxymesterone
16	4-Chlorotestosterone-17-acetate
17	Testosterone-17β-cypionate
18	1-Dehydrotestosterone benzoate
19	1-Dehydrotestosterone undecylenate

steroids found that a program rate of 9°C/min would satisfactorily separate a large number of anabolic steroids using a 12-m × 0.2-mm-i.d. (0.33-μm film thickness) HP-1 fused-silica capillary column (56). Table 16.6 lists the retention times for a number of steroids using this program and column conditions on both methylsilicone and 5% phenylmethylsilicone fused-silica capillary columns.

Steroids are usually found in three dosage forms: (1) tablets and capsules, (2) aqueous suspensions (injectables), and (3) oil solutions (injectables). A variety of oils, vitamins, plant sterols, and plant extracts have been found in exhibits suspected of containing anabolic steroids. In addition, fillers and/or caffeine have been substituted in tablets. Mixtures of anabolic steroids are also found in some samples, none of which may be the steroid(s) listed on the label. For these reasons sample preparation of the steroid products is made somewhat complex because of the various ways in which these samples are found. Sometimes it may be necessary to refrigerate the sample first to allow the oil phase and aqueous phase to separate, subsequently providing a cleaner and more efficient extraction. An analytical method consisting of extraction, TLC, UV spectra, and GCMS was devised for 13 commonly abused anabolic steroids (57). This extraction is outlined in Table 16.7.

GCIR has been used for the separation and detection of all except one pair of testosterone and its 11 esters (58). Included in this paper are the analysis preparations for these particular compounds. For more information on the analysis and identification of anabolic steroids, the reader is referred to Reference 23.

TABLE 16.6 Retention Times of Anabolic Steroids

		Retention Times (min)	
Peak Number	Steroid	HP-1	HP-5
1	Androsterone	8.73	9.84
2	19-Nortestosterone	9.23	10.38
3	Testosterone	9.79	10.98
4	Methyltestosterone	10.06	11.20
5	Norethandrolone	10.61	11.72
6	Testosterone acetate	10.82	11.93
7	19-Nortestosterone 17-propionate	11.17	12.27
8	Testosterone propionate	11.69	12.78
9	Testosterone isobutyrate	12.10	13.21
10	Clostebol	12.76	13.98
11	Stanozolol	13.32	14.81
12	Testosterone enanthate	15.10	16.51
13	19-Nortestosterone benzoate	16.11	18.23
14	Testosterone-3-benzoate	16.85	19.13
15	Testosterone 17β-cypionate	17.28	19.57
16	19-Nortestosterone-17-decanoate	17.80	20.14
17	19-Nortestosterone-17-phenylpropionate	18.07	20.96
18	Testosterone undecanoate	20.30	23.50

Source: Reference 60.

TABLE 16.7 Extraction for Anabolic Steroids

Two tablets of the solid dosage are crushed to a fine powder and added to a 10×75-mm test tube and 1 mL of methanol[a] is added
If the dosage form is a liquid injectable, 1 mL is placed in the test tube and 1 mL of methanol is added
The mixture is shaken vigorously and vortexed for approximately 30 s
The emulsion that may form is broken up by centrifuging the mixture at high speed for 2 min
If the top supernatant layer is not clear, it should be filtered through qualitative filter paper
The clear filtrate or supernatant liquid is now ready for UV, TLC, and GCMS analysis

[a] Because of the low solubility in methanol, stanozolol and oxandrolone are extracted in dimethyl formamide (DMF) and methylene chloride, respectively.

16.5 QUANTITATIVE ANALYSIS OF DRUGS OF ABUSE

The use of GC and GCMS for quantitative analysis of drugs of abuse is now a routine procedure performed in nearly every forensic drug laboratory. The chromatographic conditions are critical to the success of the method. The major characteristics of the chromatographic system are that the gas chromatographic column be thermally stable (low bleed) and inert, provide good peak shape,

and provide satisfactory resolution of the analyte and any interfering compound. Fused-silica capillary columns generally fulfill all of these requirements for quantitative analysis of illicit drugs. The excellent resolution achievable with capillary columns contributes to improved sensitivity and specificity.

The first step in the development of a quantitative gas chromatographic procedure is to establish the performance characteristics of the drug in the absence of any matrix. Precision (repeatability), linearity, limit of detection (LOD), and reproducibility all must be demonstrated. Purity of the standard should be established by an independent technique to ensure structural integrity. The linearity range should be compatible with sample and standard availability and should bracket the concentration range of the analyte. The point should be to demonstrate that the chromatographic system is linear, reproducible, and compatible with the desired drug concentration range. Peak heights or areas can be used, depending on the detector. The solvent used should be compatible with both drug analyte and internal standard (if used) and should be one that will serve as the final extraction solvent in the sample preparation. This is important to avoid possible drug–solvent interactions that could result in abnormal detector responses and/or "ghost" peaks.

A "resolution standard" should be used before any quantitative data are generated to determine whether the chromatographic system is performing acceptably. A standard should be selected that closely resembles and behaves in a chromatographically similar way to the analyte drug. Fortunately, a variety of structural analogues are usually available in drug analysis, so that an appropriate selection can be made. A standard should be selected that will assure that the desired separation is reproducible on a day-to-day, basis. For example, Figure 16.4 shows the separation of cocaine and four analogues. This mixture could be used for this purpose.

An internal standard should be used for quantification of illicit drugs. There are two purposes for choosing an internal standard. The first is to compensate for any variations in the injection of the sample and standards. The second function of the internal standard is to improve the reproducibility and buffer against any chromatographic changes that might take place during the analysis. For best results, the internal standard should be structurally similar to the drug analyte such that they have similar polarity and volatility. This will result in optimum partitioning and reproducibility.

The discussion has so far assumed that no derivatization has been necessary for achieving good quantitative data. Derivatization can serve a variety of purposes. Most commonly it is used to improve the chromatographic characteristics of the analyte and hence improve resolution and sensitivity. The ideal derivative should

1. Be easily prepared
2. Be prepared in high yield
3. Have good chromatographic characteristics
4. Be chemically stable
5. Be thermally stable

6. Improve the detector response
7. Be efficiently ionized (if MS is used)
8. Give an abundant ion current at a structurally characteristic mass that is free from ions generated by coextractants (if MS is used) (59)

The derivatization step is an added complication, which can make quantification more difficult and lessen reproducibility in some cases. It should be avoided if at all possible. Methodology for chemical derivatization of drugs for chromatographic and related analyses can be found in several comprehensive references (60–62). Readers are referred to these sources for complete information. Specific applications of derivatization can be found in the separate sections of the particular drug classes covered in this chapter.

GCMS is used routinely for quantitative analysis of drugs of abuse and quite often is used in the selective-ion-monitoring (SIM) mode. Once the fragmentation pattern is known for a particular drug and a suitable internal standard is chosen, a quantitative method using GCMS can be developed. Generally, the most abundant m/z fragments yield the best linearity and reproducibility. It is important to use the sample matrix as a blank, if possible (whether in the form of powders, tablets, or biological fluids, such as blood, serum, or urine), to assure that the analyte drug can be separated from potential interferences. Area or peak height of the selected ions is measured and provides the basis for quantification of the samples. Quantification based on isotopically labeled internal standards yields the best results, since the standards closely mimic the analyte drug chromatographically and give very similar detector responses. Isotopically labeled drug standards are available commercially and are easily obtained in small quantities without a Drug Enforcement Administration (DEA) license.

16.6 SOURCE DISCRIMINATION AND IDENTIFICATION

Often the forensic scientist is asked to compare two drug samples to show that they may have come from the same source or that they originated from the same batch of drug. Unlike legitimate pharmaceutical preparations, illicit drug samples are often contaminated with impurities and adulterants. The impurities can originate from a variety of sources, such as the precursors and chemicals used to synthesize the drug, the synthetic procedure, including incomplete reactions and side reactions, decomposition, and handling and packaging of the drug. Taking this information into consideration, it is understandable that chromatographic patterns resulting from illicit drug samples can be used to compare samples. A great deal of information can be learned about the history of the sample and the route of synthesis by these “chemical signature” analyses.

Initially, crime laboratories attempted to accomplish this type of analysis by determining the ratio of the concentrations of the parent drug to that of any adulterant or diluent present in the sample. A more successful technique, however, has been the qualitative and quantitative determination of the impurities in the

illicit sample. This has been done for heroin (63), amphetamine (64), methamphetamine (65), hashish (66), and phenmetrazine and morphine (67). The ratio of the impurity to the main drug, rather than its absolute concentration, is used. This is done in order to eliminate the effect of added adulterants and diluents (64). This procedure has been used for the comparison of methamphetamine, heroin, and cocaine specimens.

Cocaine, for example, is a naturally occurring alkaloid that can be extracted from the leaves of *Erythroxylon coca.* Cocaine purity can vary, depending on the extraction and purification process. The amounts and varieties of related alkaloids available for sample comparisons are also dependent on the source of the leaves and the extraction and purification process.

The amount of sample used in the analysis should be as large as the methodology will permit. The following procedure can be used as an example (68):

1. Accurately weigh an amount of sample equivalent to 50 mg of cocaine into a glass-stoppered test tube.
2. Add 1.0 mL of $CHCl_3$ containing 0.5 mg/mL of octacontane and octacosane.
3. Add 1 mL of BSA (bistrimethylsilyacetamide).
4. Heat at 60°C for 15 min.
5. Inject sample.

Figure 16.8 shows the separation of a typical uncut cocaine sample as a result of this procedure. Some of the components present, besides cocaine, are methylecgonine, ecgonine, and benzoylecgonine. These compounds can be used for sample comparisons, but one must be cautious, since they can be formed from decomposition. Also present are *cis*- and *trans*-cinnamoylcocaine. These particular compounds can be used to significant advantage in comparisons, since their ratio and concentrations can vary significantly with geographical origins of the coca plant. Cocaine sample differentiation requires the determination of synthetic or natural origin. Synthetic samples are characterized by the presence of optical isomers, certain diastereoisomers, and other byproducts and chemical residues. Samples derived from the coca plant are characterized by the presence of certain natural products and their derivatives, and residual chemicals. The rationale for developing cocaine-profiling methodology has been described and the cocaine signature procedures in use at the United States Drug Enforcement Agency's Special Testing and Research Laboratory have been reviewed (69).

SPME, coupled with GCMS, has been used to characterize impurities in illicit methamphetamine samples placed in a sealed headspace vial (70). This method works well for generating material "fingerprint" profiles in methamphetamine samples. The detection and characterization of increased points of comparison by this method as compared to a conventional solvent extraction provides more detailed chemical signatures for both intelligence and operational information.

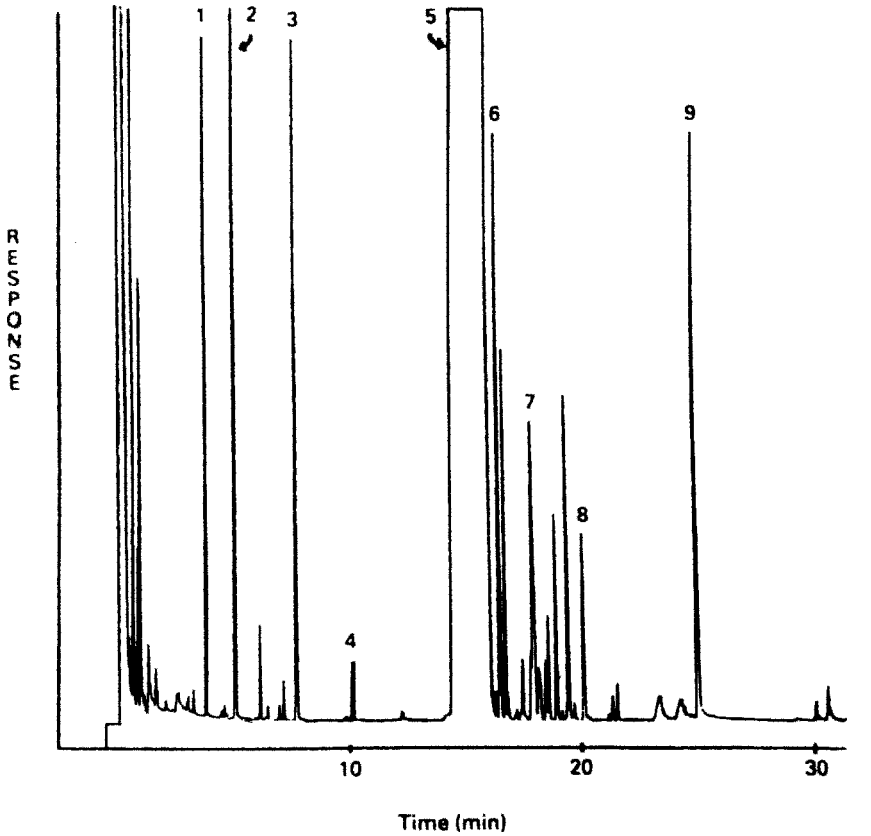


FIGURE 16.8 Separation of impurities in an illicit cocaine sample: 1, ecgonine methyl ester TMS; 2, ecgonine di-TMS; 3, *n*-C18 internal standard; 4, tropacocaine; 5, cocaine; 6, benzoylecgonine TMS; 7, *cis*-cinnamoylcocaine; 8, *trans*-cinnamoylcocaine; 9, *n*-C28 internal standard. Oven temperature program: initial temperature 170°C, initial hold for 1 min, heating rate 5°C/min, final temperature 270°C, final hold for 10 min. (Reprinted with permission from Reference 8.)

16.7 CLANDESTINE LABORATORY ANALYSIS

The analysis of materials seized from clandestine drug laboratories falls into the realm of the responsibilities of the forensic scientist. Because this is essentially a "chemical investigation," the forensic scientist plays a major role in all phases of the investigation. On the basis of the information gathered, the scientist's responsibilities may include the following:

1. Formulation of an opinion as to what drug is being synthesized
2. Determination of synthesis route
3. Estimation or determination of production capability

4. Projection of synthesis time
5. Determination of the degree of hazard to be encountered
6. Determination of the function of laboratory apparatus
7. Preservation and collection of evidence
8. Analysis of evidence submitted to laboratory
9. Aiding clean-up, removal, and destruction of chemicals
10. Acting as scientific advisor to prosecutors and investigators
11. Offering expert testimony

Laboratory analysis in this type of crime normally focuses on the identification of drugs and precursors to determine the synthesis route(s) and to estimate the production capability. GC is a highly effective tool in these analyses. Since many samples may contain complex mixtures of precursors, impurities, and byproducts, high resolution is essential and the use of capillary columns are recommended.

The most common drugs clandestinely manufactured in the United States are methamphetamine, amphetamine, MDA and its analogs, PCP, LSD, and methaqualone. The Leuckart reaction has been the most popular method for synthesizing illicit amphetamine in the United States, while illicit methamphetamine has been produced primarily by reductive amination using benzylmethylketone and methylamine. PCP is commonly prepared using precursors such as piperidine, cyclohexanone, and phenyl magnesium bromide. All of these clandestinely manufactured drugs have several different synthesis routes that use different reagents and precursors.

GC has been applied in the analysis of clandestine samples by separating the different components and identifying the precursors and chemicals that have been used in the synthesis. Mass spectrometry has been almost universally employed as the detector in this type of analysis, since unequivocal identification of the components is essential. As an example, Figure 16.9 shows the total-ion chromatogram (TIC) from a 2-mg sample of vegetation (mint) adulterated with PCP thermally desorbed directly at 85°C for 5 min. In addition to PCP, the precursors used in the synthesis, cyclohexanone and piperidine, are easily detected. This is a rather unique application involving thermal desorption of the chemicals from the vegetation directly into the injection port of the gas chromatograph. More commonly, a solvent extraction is performed and a sample of the liquid extract is injected into the gas chromatographic column for separation and detection of the precursors.

In contrast to legitimate drug formulations, illicit drug samples are often contaminated with impurities as a result of inadequate purification procedures. As previously discussed, gas chromatographic patterns originating from these drugs contain valuable information about the drug and its synthesis route. Some basic work has been done regarding the nature of contaminants encountered in the different synthesis of drugs of abuse (65).

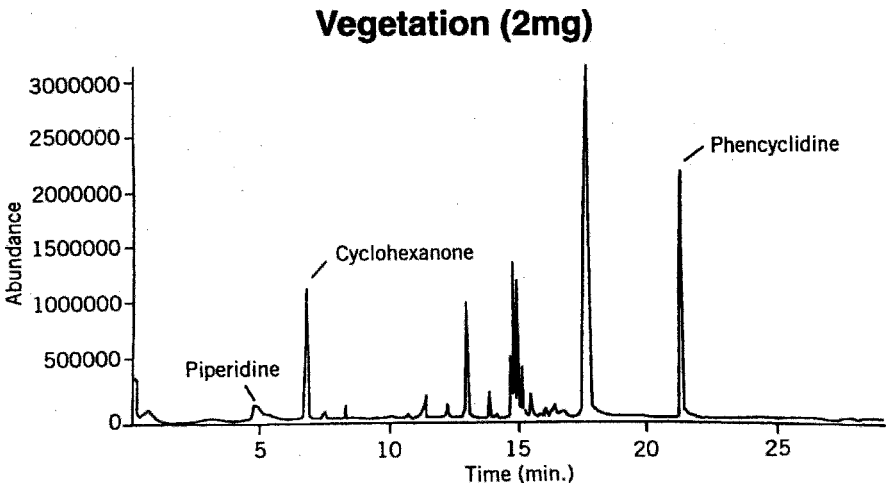


FIGURE 16.9 Total-ion chromatogram resulting from volatiles of a 2-mg sample of vegetation (mint) adulterated with phencyclidine (PCP) desorbed directly into the gas chromatographic injection port with a short-path thermal desorption unit at 85°C for 5 min. GCMSD conditions: 12-m HP-1 × 0.20-mm-i.d. × 0.33-μm film; −10 to 250°C at 10°C/min; injection = 250°C. (Courtesy of New Jersey State Police.)

PART 3 GAS CHROMATOGRAPHY IN FORENSIC TOXICOLOGY

16.8 APPLICATIONS OF GAS CHROMATOGRAPHY IN FORENSIC TOXICOLOGY

16.8.1 Drug Analysis in Biological Fluids and Tissues

Forensic toxicology is the application of toxicology for legal purposes. The classic example is postmortem toxicology, where specimens from deceased individuals are analyzed to determine whether compounds that were found were a cause of or a contributing factor to the death of the victim. This type of analysis involves detection, identification, and quantification of an array of toxic chemicals and drugs (and metabolites), including alcohol, poisons (and metabolites), and other chemicals, such as solvents and gases. Forensic toxicology also includes the screening of drugs, including alcohol, for the determination of whether someone is under the influence of a particular drug while driving a motor vehicle. Some forensic laboratories are also asked to perform workplace testing of employees and police officers.

Samples submitted by law enforcement personnel to the forensic toxicology laboratory for analysis normally include blood, urine, brain, kidney, and bile and are generally removed by the pathologist at autopsy. In driving under the influence (DUI) cases, blood and urine from the suspect are routinely gathered by the arresting officer either at the police station or a hospital. Other sample types

that the forensic toxicology laboratory may encounter include stomach contents, foodstuffs or other drug tablets and capsules found near the victim. A vast array of toxins must be included in the screening protocols; the majority of compounds are drugs, and volatiles such as alcohol.

GC is applied in a variety of ways and is one of the most important separation techniques in this particular area. GC provides the retention time or retention index (RI) of an unknown substance that can be used for its identification. GC is routinely utilized to separate the analyte from endogenous interferences for more specific identification via mass spectrometry and can also be used to provide quantitative information about the drugs present. The following applications focus on the identification and quantification of drugs and volatiles in biological fluids by GC.

16.8.1.1 Sample Preparation

The process of screening for drugs of abuse can be divided into two stages: sample preparation and analysis of the sample. The initial step in screening for drugs of abuse is to separate the drug of interest from the biological matrix. This first involves a sample pretreatment step commonly involving dilution of samples such as plasma, serum, and urine. Whole blood can be sonicated and diluted, while tissues are usually treated by either protein precipitation or enzymic digestion. When analyte drugs are present in a conjugated form, deconjugation is required. The use of β-glucoronidase for enzymic hydrolysis of samples is the preferred procedure for the analysis of analytes such as benzodiazepines and morphine. The main purposes for sample pretreatment are the following (71):

1. Release of drugs from the biological matrix
2. Removal of proteins and particulate matter, which would interfere with further analysis
3. Adjustment of the pH, ionic strength, and concentration of the sample to allow optimum extraction efficiencies

After proper pretreatment of the sample, extraction of the drug from the matrix must be completed. Liquid–liquid extraction procedures have been used in past methods for extraction of drugs of abuse and are used today in some protocols, however, solid-phase extraction (SPE) has gained popularity in recent years because it uses fewer solvents and more samples can be extracted at one time. Presently, many types of SPE materials are commercially available for extraction of drugs. Some contain as many as three different solid phases for extracting acidic, basic, and neutral drugs. When developing an SPE procedure for drug screening each step must be carefully optimized to gain maximum recovery of the particular drug. Following are some of the many factors that affect the recovery of a drug during an SPE:

1. Selection of sorbent
2. pH of sample

3. Flowrate of the sample and eluent passing through the column or disk
4. Properties and volume of solvent wash
5. Properties and volume of solvent eluent
6. Proper pH and type of buffer

Many SPE methods and procedures for the extraction of drugs of abuse have been published in the literature and by manufacturers. These have included automated methods that use robotic systems. Comprehensive reviews have appeared that specifically discuss the SPE of abused drugs in toxicological samples (71,72).

16.8.1.2 Screening for Drugs of Abuse

Analysis for drugs of abuse in forensic toxicology is similar to the methodology used in the clinical laboratory, except the matrices encountered in forensic samples are more varied and the purposes are different. Clinical analyses are generally conducted for diagnosis purposes, while the forensic analyses are medicolegal by nature.

The National Institute on Drug Abuse (NIDA) guidelines (73) have become the standard for drug testing in laboratories that conduct workplace testing for federal agencies. This standard is increasingly demanded of private laboratories as well and is serving as a model for forensic laboratories performing analyses for drugs of abuse in biological fluids. Analysis generally begins with a screening test. This may be an immunoassay, such as enzyme immunoassay (EIA), enzyme multiplied immunoassay technique (EMIT), or radioimmunoassay (RIA), or may encompass an array of chromatographic screening techniques, such as GC, TLC, or HPLC. The confirmation test is almost always GCMS or LCMS; however, GCMS/MS and LCMS/MS are being used more frequently.

Most toxicological drug screening has been done in the past on conventional packed columns with the stationary phase coated onto inert supports (44,74). Forensic toxicologists because of the higher separation efficiency, resolution, and sensitivity now exclusively use fused-silica capillary columns. Extensive evaluations in different laboratories have shown that dimethylsilicone is the preferred phase when using capillary columns for screening drugs in forensic toxicology. Most laboratories use a combination of dimethylsilicone and phenylmethylsilicone (5–50%) capillary columns for the screening of drugs and poisons. The added polarity of the phenylmethylsilicone phase can help solve some separation problems not feasible with short (12–15 m) methylsilicone capillary columns.

Forensic toxicology laboratories employ various drug-screening procedures, which use capillary columns and detectors such as the FID and nitrogen–phosphorus detector (NPD), with mass spectrometry generally the detector of choice for confirmation. Figure 16.10 shows the separation of a drug standard mixture on a fused-silica capillary column (12 m × 0.2-mm i.d.) consisting of cross-linked dimethylsilicone with helium as the carrier gas and an FID. The temperature program and chromatographic conditions are shown in the figure. Extracts from urine, blood, or other samples can be screened on this column and

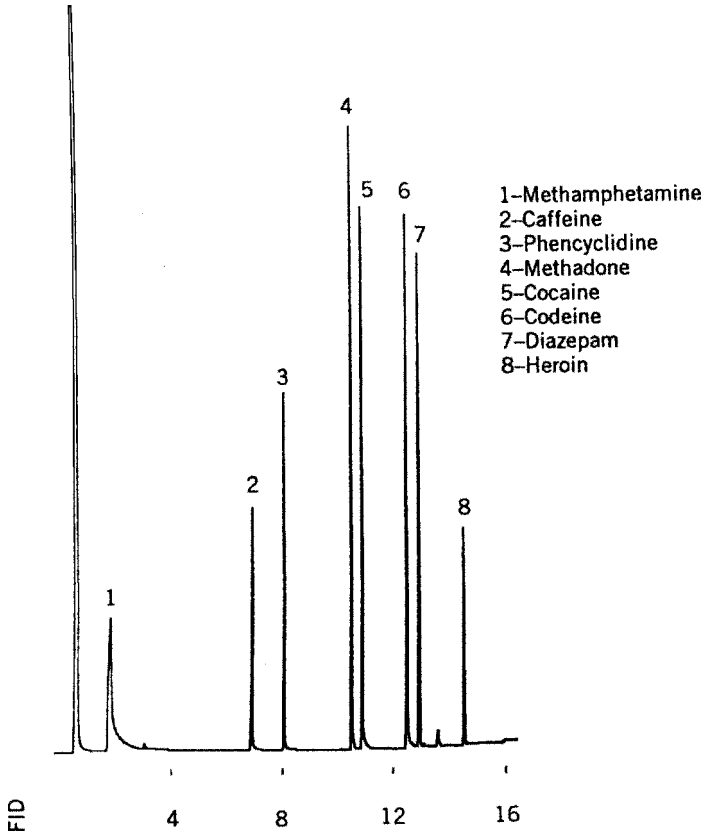


FIGURE 16.10 Separation of methamphetamine, caffeine, PCP, methadone, cocaine, codeine, diazepam and heroin. GCFID conditions: 12-m HP-1 × 0.2-mm-i.d. × 0.33-μm film; 140°C (1 min) to 260°C at 10°C/min; injection = 270°C, detector = 300°C; split ratio = 20/1. (Courtesy of New Jersey State Police.)

comparing the retention times can tentatively identify unknown drugs. The same standard mixture (diluted 1000-fold) is shown in Figure 16.11 separated on a similar column but using an NPD. The responses are different for the compounds on both detectors, but the retention data can be used for preliminary identification. Once the sample is screened, the extract can then be confirmed by using similar chromatographic conditions with GCMS.

Lillsunde and Korte (75) previously reported a screening procedure covering 300 substances, including drugs of abuse and metabolites, in which they used a combination of packed and capillary columns. Wide-bore capillary columns have also been used successfully for the screening and confirmation of drugs in forensic toxicological samples (76).

In 1981, the Committee for Systematic Toxicological Analysis of the International Association of Forensic Toxicologists also recommended that retention

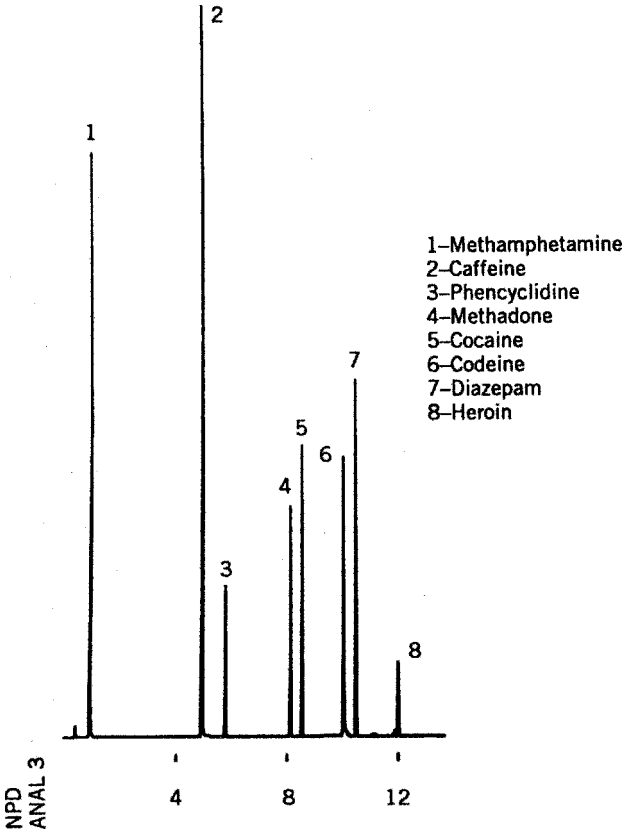


FIGURE 16.11 Separation of methamphetamine, caffeine, PCP, methadone, cocaine, codeine, diazepam, and heroin. GCNPD conditions: 12-m HP-1 × 0.2-mm-i.d. × 033-μm film; 140°C (1 min) to 260°C at 10°C/min; injection = 270°C, detector = 300°C; split ratio = 20/1. (Courtesy of New Jersey State Police.)

behavior be expressed in terms of Kovat's retention indices (RIs) (77). The interlaboratory standard deviation of RIs at that time on SE-30 or OV-1 packed columns was about 15–20 RI units, so that a search window of about 50 RI units had to be taken into account when trying to identify an unknown compound (44). In 1990, J.-P. Franke et al. (78) suggested using a carefully selected secondary standard drug mixture to improve the interlaboratory reproducibility of RI values, which even under vastly different operational conditions allows a much better search window than that which must be applied when using alkane or substituted alkane homologues. Table 16.8 lists two test mixtures suggested for use with capillary columns. Before starting a gas chromatographic study to collect or use retention indices, the authors recommend the chromatographic system should be checked by means of one of these test mixtures with regard to quality of the column separation efficiency and detection sensitivity.

TABLE 16.8 Reference Drug Mixtures for Acidic, Basic, and Neutral Drugs for the Determination of Retention Indices (RI)

Mixture A: Acidic and Neutral Drugs	RI	Mixture B: Basic and Neutral Drugs	RI
Ethosuximide	1205	Amphetamine	1125
Ethinamate	1365	Ephedrine	1365
Barbital	1489	Benzocaine	1545
Aprobarbital	1618	Methylphenidate	1725
Secobarbital	1786	Diphenhydramine	1870
Phenobarbital	1953	Tripelenamine	1976
Heptabarbital	2055	Methaqualone	2135
Primidone	2250	Trimipramine	2215
Phenylbutazone	2367	Codeine	2375
Bis(2-ethylhexyl)phthalate	2507	Nordiazepam	2490
Prazepam	2648	Prazepam	2648
Clonazepam	2823	Papaverine	2825
		Haloperidol	2930
		Strychnine	3116

Source: Reprinted with permission from Reference 79.

These test mixtures cover a broad range of RI values and the chromatographic system should be able to detect 100 ng of each component with a good separation of all substances with acceptable peak shape. An example of a separation of mixture B is given in Figure 16.12 (amphetamine, trimipramine, and haloperidol are not shown). The calculation of RIs for individual unknown substances can be accomplished by comparing retention times of the unknown substance to the retention times and retention indices of the "bracketing" standards (80).

Opiates are a major group of abused drugs for which the forensic toxicology laboratory routinely tests. Morphine, codeine, and 6-monoacetylmorphine (6-MAM) are common drugs and metabolites that are routinely identified in drug overdose specimens. Morphine is present in many prescriptions for treatment of pain and cough suppression and is also a metabolite of codeine and ethylmorphine. Because of this, morphine's presence cannot be used solely for identifying heroin use. It has been reported that 6-MAM can be used as an indicator of heroin use, since heroin is metabolized in the body first as 6-MAM and then to morphine (81). Figure 16.13 shows the total-ion chromatogram (TIC) of an extract of urine (5 mL) from a driver arrested for being under the influence of drugs. This particular sample is a typical example of a urine containing multiple drugs and metabolites. The presence of methadone, cocaine, codeine, and morphine, and several metabolites, including 6-MAM are shown. Gas chromatographic methods for the determination of 6-MAM in various body fluids have been reported (see Table 16.9). These procedures have used a variety of methods and have included derivatization and GCMS.

Cocaine is metabolized into two major metabolites, benzoylecgonine (BZE) and ecgonine methyl ester (EME), and to a lesser extent into other metabolites

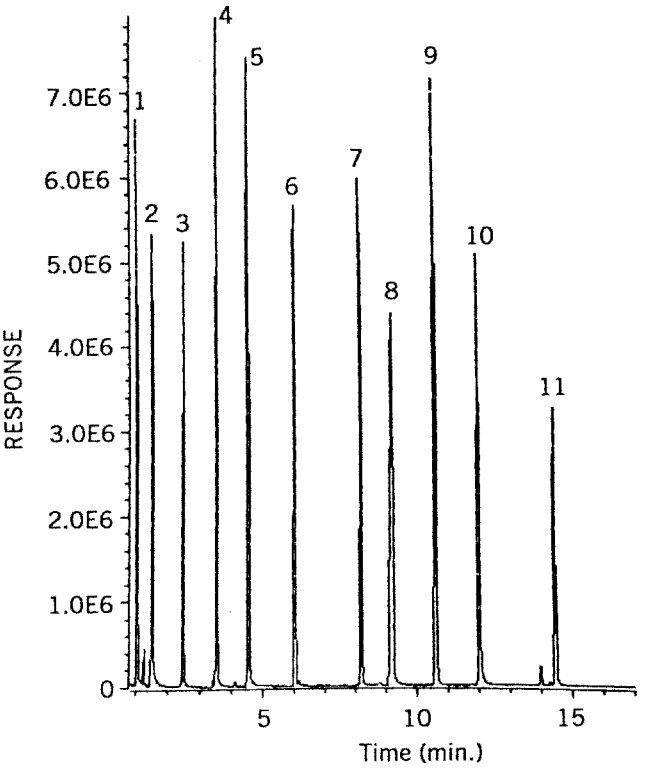


FIGURE 16.12 Separation of reference mixture B (amphetamine, trimipramine, and haloperidol are not shown): 1, ephedrine; 2, benzocaine; 3, methylphenidate; 4, diphenhydramine; 5, tripelenamine, 6, methaqualone; 7, codeine; 8, nordiazepam; 9, prazepam; 10, papaverine; 11, strychnine. GCMSD conditions: 12-m HP-1 fused-silica capillary column × 0.2-mm-i.d. × 0.33-μm film; oven = 190°C (2 min) to 300°C at 9°C/min, injection = 270°C; split ratio = 20/1.

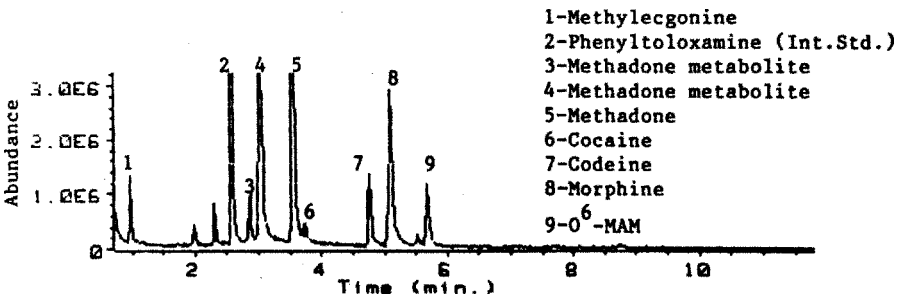


FIGURE 16.13 Total-ion chromatogram of a urine extract. GCMSD conditions: 12-m HP-1 fused-silica capillary column (0.2-mm-i.d. × 0.33-μm film); oven = 200 to 260°C at 15°C/min, injection = 270°C; split ratio = 20/1. (Courtesy of New Jersey State Police.)

TABLE 16.9 Gas Chromatographic Methods for Determination of Drugs and Metabolites in Biological Fluids

Drug	Derivative	References
6-MAM	Propionyl ester	82
	MBTFA	83
	Trimethylsilyl	84
Morphine, codeine, and 6-MAM	Acetic anhydride (deuterated)	85
Codeine and morphine	Silylation	86
	Pentafluoropropionylation	87
	Trifluoroacetylation	81,88
	Heptafluorobutyrylation	89
	Acetylation	90
Cocaine and BZE	Butyl ester	91
	Trimethylsilyl	92
	Propyl ester	93
	Pentafluoropropionylation	94
	Hexafluoroisopropylation	95
	Hexachloroformate	96
Amphetamine and methamphetamine	Heptafluoro	75
	Heptafluorobutylation	97
	Trifluoracetic anhydride	98
	Trichloroacetylation	99
	N-Trifluoroacetyl-L-prolyl chlorides	100
	MTBSTFA	101
	4-Carbethoxyhexafluorobutyryl chloride	102
	Perfluorooctanoyl chloride	103
Barbiturates	DMF dipropylacetal	104
	TMAH	107
THC and metabolites	3-Pyridine diazonium chloride	111
	Hexaflurooisopropyl/pentafluoropropionyl	112
	Pentafluorobenzyl bromide	113
	MSTFA	114
	tert-Butyldimethylsilyl	115

such as norcocaine, and ecgonine. In addition, cocaine is unstable in aqueous solutions, including urine, above pH 5, and also in blood. All of these factors make the interpretation of cocaine concentrations difficult. The normal procedure for analysis of biological fluids generally involves the identification of cocaine in addition to its metabolites. Gas chromatographic procedures for the detection of cocaine and its metabolites in biological fluids are numerous and diverse. Cocaine and EME can be identified by GCMS without the need for derivatization; however, methods for the determination of BZE and ecgonine usually require some type of derivatization. Table 16.9 lists some examples of derivatization used to chromatograph these metabolites. The majority of the methods for the

identification of cocaine and its metabolites are done on short, narrow-bore, fused-silica capillary columns coated with methylsilicone or phenylmethylsilicone and chromatographed using programmed-temperature runs. GCMS is universally used for the confirmation of the drugs.

Amphetamine and methamphetamine are the most commonly abused central nervous stimulants (CNS) and consequently are often found in toxicological specimens. Diethylpropion, phentermine, and phendimetrazine, as well as MDA and MDMA, are also found in biological samples. Generally, these compounds can be screened using capillary columns with relatively low oven temperatures (150°C) and FID. Nitrogen–phosphorus detectors can be used to increase the sensitivity and reduce background peaks when screening for low levels of amphetamines in biological samples (see Figures 16.10 and 16.11). Like most other drug analyses, confirmation of abused CNS drugs can be accomplished by GC/MS.

When screening for CNS type drugs the free base is normally chromatographed and detected with the use of the FID and/or the NPD. When analyzed without derivatization, however, peak tailing and resulting sensitivity problems are often encountered. To correct these problems, a variety of derivatization reagents have been employed. Derivatization is almost always used in gas chromatographic methods to confirm the identity of the amphetamines with GCMS because the free-base amphetamines do not give high-molecular-weight ions, which results in mass spectra that are not very discriminating. The most common derivative for amphetamine and methamphetamine is the trifluoroacetyl or the trichloroacetyl derivative. Table 16.9 lists some derivatization procedures that have been used to chromatograph these compounds, including chiral reagents.

Methods for identification of amphetamine and methamphetamine in urine have been employed using GC/Fourier transform IR (GC/FTIR) spectroscopy (104) however these are not widely used. These methods have provided identification of the amphetamines and metabolites at the low picogram levels. Developments in cryogenic sample deposition for GC/FTIR spectroscopy have allowed the highly selective ability of IR spectroscopy to be used for identification and quantification of these drugs.

Barbiturates are commonly detected in forensic specimens from both overdose cases and driving while intoxicated (DWI) cases. Butalbital and phenobarbital are two of the most commonly abused barbiturates. The formation of the N, N'-dimethylderivatives of the 5,5′-disubstituted barbiturates is a common procedure used in many clinical and forensic laboratories to reduce the adsorption on the column and peak tailing. Mule and Casella (105) reported a detection limit of 20 ng/mL in human urine using GCMS with this procedure. The barbiturates have been detected using other derivatization techniques, including the use of on-column derivatization with trimethylanilinium hydroxide (TMAH) (see Table 16.9).

The 1,4- and 1,5-benzodiazepines are among the most prescribed tranquilizers, hypnotics, and muscle relaxant drugs available today. Hence, they are frequently abused and often found in DWI or drug overdose cases in combination with other drugs or alcohol. In addition, benzodiazepines have been identified as one

of the classes of drugs used for the purposes of "drugging" unsuspecting victims and raping them while they are under the influence of these substances often combined with alcohol. Flunitrazepam, also known by several slang/street names including "roofies" and others, was the particular drug, which caught the attention of the media in "drug-facilitated sexual assaults" labeled as "date rape." Methods have been described to determine this drug at low concentrations by GCMS in biological fluids (108,109). Benzodiazepine concentrations are usually higher in the urine than the blood and the metabolites are detectable for longer times, so most procedures are designed to detect these compounds and their metabolites in the urine.

Benzodiazepines and their metabolites are normally excreted as the glucuronide conjugates and require either acid or enzyme hydrolysis for good recovery. Hydrolysis of the benzodiazepines yields the corresponding benzophenone, which can be identified by GCMS and related back to the parent benzodiazepine. In some cases, however, the specific benzodiazepine cannot be identified because some benzodiazepines yield the same benzophenone after acid hydrolysis. In addition, some benzodiazepines yield the same metabolites. For example, diazepam and chlordiazepoxide both metabolize to desmethyldiazepam and oxazepam. To eliminate this problem and lower the limit of detection, it is possible to derivatize the benzodiazepines using BSTFA to form their trimethylsilyl derivatives.

ECD has been used for the detection of this class of drugs because of the increased sensitivity over the FID or NPD to detect the lower therapeutic doses of certain benzodiazepines, such as triazolam and alprazolam. Triazolam and alprazolam give well-defined peaks as underivatized drugs, while anhydrides (trifluoroacetic, pentafluoropropionic, and acetic) have been used to form the esters of the metabolites. The acetic anhydride derivatives have also been very successful producing good yields with few apparent decomposition products.

The identification and quantification of lorazepam in blood is of forensic interest. A specific and sensitive analytical method is required because of the low concentrations normally detected in blood. Lorazepam has been quantified by GCECD without derivatization and after hydrolysis of the parent drug to the benzophenone. Trimethylsilyl and heptafluorobutyryl derivatives have also been used for quantification of lorazepam in biological fluids. A successful application of GC/negative-ion chemical ionization mass spectrometry (NICIMS) for the analysis of lorazepam and triazolam in postmortem blood has reported a detection limit of 0.5 ng/mL (110). This method used a fused-silica capillary for the detection of PCP. Several of these methods have incorporated the identification of PCP with a general gas chromatographic column (DB-1, 15-m $\times$ 0.25-mm-i.d., 0.1-μm film thickness) coupled directly to the ion source of a mass spectrometer set up in the negative chemical ionization mode with methane as the reagent gas.

Phencyclidine (PCP) is rapidly metabolized in the body and excreted in the urine as several hydroxy metabolites and the parent drug. Many gas chromatographic methods have been developed for screening of different drugs, such as that shown

in Figure 16.10. Most procedures use short capillary columns and either FID or NPD for screening and mass spectrometric methods for confirmation.

There are numerous reports for the gas chromatographic determination of THC and its metabolites, 11-nor-Δ-9-tetrahydrocannibinol-9-carboxylic acid (THC-COOH) and 11-hydroxy-Δ-9-tetrahydrocannibinol (11-OH-THC) in urine and blood. THC is not normally found in urine, so it must be determined in blood at levels around 2–4 ng/mL. The TMS derivative is the most widely used derivatization procedure with GCMS for the determination of cannabinoids. In addition to the obvious advantages of derivatizing the THC metabolites, the acidic constituents of cannabis must be derivatized because they can easily decarboxylate above 80°C. Almost all gas chromatographic procedures today use fused-silica capillary columns for this analysis. Determination of THC in blood is routinely done in forensic toxicological samples, and the detection and quantification of the two THC metabolites in urine is a routine procedure for proof of cannabis use in workplace testing. Several of the procedures used for this type of analysis are listed in Table 16.9.

Lysergic acid diethylamide (LSD) is rapidly metabolized in the body and hence less than 1% is excreted unchanged. This makes LSD very difficult to identify since dosages are in the 100-μg range. In addition, LSD must be derivatized, usually as the trimethylsilyl derivative, which degrades very rapidly in the presence of water. Also, samples must be stored away from sunlight, since this also adds to the degradation. Short fused-silica capillary columns (12–15 m) GCMS, and derivatization with BSTFA have been successful in detecting LSD in biological fluids. Using the trimethylsilyl derivative, a detection limit of 10 pg/mL in urine has been reported (116). Even with these procedures, the column must be conditioned to neutralize excess silicic acid, which reduces sensitivity. The *N*-trifluoroacetyl derivative of LSD has been used along with GC/negative chemical ionization mass spectrometry to measure less than 100 pg/mL of LSD in plasma (117).

There has been a dramatic increase in the use of gamma-hydroxybutyrate (GHB) and related compounds. Because of their current popularity as recreational drugs of abuse and their use in "drug-facilitated sexual assaults," crime laboratory submissions are on the rise for this particular class of substances. Early gas chromatographic analyses were developed for the measurement of endogenous GHB in tissues. Samples were heated in the presence of mineral acids, converting GHB to GBL, with detection limits in the order of 0.2 mg/L. More recent GCMS methods have been developed for quantifying GHB in human plasma and urine as low as 0.1–2 mg/L by converting GHB to GBL. GCMS methods for the direct measurement of GHB in urine and blood without GBL conversion have been developed recently with detection limits of 0.5–2 mg/L (118,119).

Two other groups of drugs encountered in casework samples are the antipsychotics and antiinflammatory drugs. The phenothiazines, such as chloropromazine and its analogs, and the tricyclic antidepressants, such as amitriptyline, nortriptyline and imipramine, account for the majority of antipsychotic drugs that are normally detected. The most frequently used procedures for the detection

of these drugs are GCFID, GCNPD, and GCECD; NPD being the method of choice. Aspirin, acetaminophen, ibuprofen, ketoprofen, and indomethacin are the most widely detected antiinflammatory drugs detected in the forensic toxicology laboratory. Generally, GC analysis of these particular drugs involves the formation of the methyl derivative with iodomethane and potassium carbonate, not with TMAH.

16.8.1.3 Analysis of Unconventional Samples

Historically, blood, urine, organs, and other tissues have been the common forensic specimens chosen for analysis of drugs of abuse and poisons. More recently, however, interest in unconventional samples such as hair, nail, saliva, and sweat has increased in the forensic field. This interest has largely been due to the several potential advantages over current drug methodologies that employ body fluids. These samples are noninvasive, and samples such as hair and nails retain drugs over long periods of time, providing valuable information on the degree and pattern of drug use.

The analysis of hair for drugs of abuse has received considerable attention recently. Many laboratories are now reporting the ability to confirm the presence of drugs of abuse and drug metabolites in human hair. Among the drugs confirmed are cocaine, opiates, amphetamines, and PCP using a combination of techniques such as immunoassay, GCMS, and MS/MS. For quantitative analysis, it is difficult to obtain a representative sample because the drugs are not uniformly distributed along the shaft of the hair or between hairs. In addition, other considerations complicate the analysis, such as extraction of the drug and environmental contamination.

Despite these difficulties, laboratories have been successful in detecting and quantifying drugs of abuse in hair by automated SPE, GCMS and GC/CIMS (120). Welch et al. (121) have described the development of a standard hair reference material and a method for quantifying cocaine, benzoylecgonine, codeine, and morphine using GC/MSD. The method used a 1- to 5-μL splitless injection onto a DB-5, 12-m × 0.20-mm-i.d. fused-silica capillary column (0.33-μm film thickness). Selected-ion monitoring with isotopic dilution was used for quantification. The limit of detection was reported to be at 0.5 ng/mg of hair. Extraction of hair from a cocaine user with 0.1 N HCl at 45°C overnight gave a high recovery of both cocaine and benzoylecgonine.

A single exposure to GHB a month after a case of sexual assault was confirmed by analyzing the hair using GCMS/MS (122). The hair was rapidly (2 mins) decontaminated with dichloromethane, and then the hair shaft was cut into 3-mm segments, incubated overnight in 0.01 N NaOH in the presence of GHB-d_6, followed by neutralization and extraction in ethyl acetate under acidic conditions. GHB was detected by GCMS/MS after derivatization with BSTFA + 1%TMCS.

Since saliva levels of many drugs correspond to the concentration in plasma, interest has grown in this specimen for use in forensic investigations. One of the advantages of saliva as a sample is the minimal requirement in sample preparation. Drugs in saliva are usually extracted by liquid–liquid extraction or by SPE.

Deproteinization with methanol and perchloric acid prior to extraction may be necessary. The use of saliva in the forensic detection of drugs has been reviewed by Caddy (123). Included in this report are a number of systems and references for the gas chromatographic analysis of drugs in saliva.

Nail analysis has not been widely utilized in forensic toxicology, but some methods have been reported using GC (124). Nail samples receive similar treatment to hair samples for extraction of the drug.

The analysis of sweat for detecting drugs is rarely performed because it is extremely difficult to estimate drug levels. Sweat samples are collected on gauze or cotton by wiping the surface of the skin, eluting with water, and extracting by liquid–liquid extraction. The detection of cocaine, morphine, cannabinoids and amphetamine has been reported in sweat as well as drugs in perspiration stains (125).

A novel "patch" approach of sweat collection has been introduced recently for testing of drugs. Although not yet cleared for drug-testing purposes, the adhesive patch is approved for collecting perspiration, and may eventually prove to be a viable technique. The device consists of an adhesive layer on a 2×3-in. transparent film that adheres to the skin. The patch contains an adsorbent pad in its center that collects sweat as it exits the body. The device is intended to be worn for up to 2 weeks at a time, after which the collected sweat residue is removed by a simple extraction procedure. So far, the sweat patch has been effective in monitoring low levels of cocaine and heroin.

Insect larvae have also been a source for drug detection in death investigations. For example, cocaine and benzoylecgonine have been determined in insect larvae found on a decomposed body using GCMS (126).

16.8.2 Analysis of Ethanol and Other Volatiles

Gas chromatography is the most widely used technique for identification and quantification of ethanol in biological fluids. The fact that ethanol has a low molecular weight and high vapor pressure and can be chromatographed easily on polar liquid phases makes GC the technique of choice. In addition, ethanol and other volatiles can be quantified and identified simultaneously. Blood alcohol analysis in driving while intoxicated (DWI) cases is one of the most often requested analysis in the forensic toxicology laboratory. Since the relationship between blood alcohol concentration (BAC) and driving impairment is well established, laws have mandated a BAC level above which driving is considered unsafe and prohibited.

BAC levels are also necessary for death investigations, since fatal accidents may involve alcohol as a contributing factor. Deaths due to acute or chronic effects of alcohol alone or in combination with other drugs may also require determination of BAC levels. In addition, blood or urine determination may also be part of the protocol for many workplace drug-testing programs.

Many other toxic volatile liquids and gases in addition to ethanol are abused or are involved in death investigations, including fatal accidents. GC is also used for their determination. For example, methanol (wood alcohol), isopropanol

(rubbing alcohol), ethylene glycol (antifreeze), solvents such as toluene (glue), acetone, 1,1,1-trichloroethane, Freon, volatiles from commercial products, particularly butane, and nitrous oxide have all been determined by GC in forensic investigations. In addition, fire-related deaths require the analysis of body fluids for carbon monoxide, hydrogen cyanide, and nitriles. Gas chromatographic procedures for all these compounds and others have been described. Table 16.10 lists some volatile liquids and gases with corresponding references to specific gas chromatographic methods used for their determination in forensic applications.

GC of the nonhalogenated volatiles is typically done on the same system as ethanol. This procedure normally uses an FID and polar liquid phase such as Carbowax. The temperature may be modified from the routine blood alcohol procedure and a thick-film capillary column is recommended for best resolution of volatiles. The analysis of chlorinated and fluorinated volatiles requires the use of ECD since the sensitivity of FID does not meet the requirements of forensic samples. GCMS procedures have also been applied to the screening of volatiles in biological specimens, but even these procedures are sometimes lacking necessary sensitivity and may require specialized techniques, such as enhanced mass resolution. For further information on the analysis of volatile substances in toxicology the reader is referred to Reference 127. A book has been published which provides reliable gas chromatographic retention indices for volatile substances frequently encountered in analytical toxicology (128).

16.8.2.1 Determination of Ethanol in Biological Fluids

Gas chromatography is used to determine ethanol in blood from DWI suspects and in autopsy specimens, which may include blood, urine, vitreous humor, spinal

TABLE 16.10 Examples of Toxic Volatiles and Gases Determined by GC in Forensic Investigations

Volatile	Reference
Acetonitrile	129
Acrylonitrile and acetonitrile	130
Benzene	131
Butanol	132
Carbon monoxide	133
Cyanide	134
Cyclopropane	135
Kerosene	136
C_2ClF_5 and $CHClF_2$	137
Phenol	138
Enflurane	139
Methanol	140
Propane and ethyl mercaptan	141
Paint thinner	142
Toluene	143
Tricholoroethlene	144

fluid, and organs such as brain tissue. Urine samples are often analyzed for ethanol, but the variation in conversion of urine to blood ethanol values leaves the result of little forensic value. Saliva as an alternative specimen for alcohol determination in the human body is also getting some attention in more recent studies (145)

Blood samples are normally collected into an evacuated tube containing preservatives and anticoagulants such as sodium fluoride and potassium oxalate. The collection tube should be 75% or more filled with blood to reduce the risk of any loss of volatiles. It should also be properly sealed, labeled, and stored under refrigeration. If the tube is improperly resealed after analysis or if it is not refrigerated for extended periods of time, volatiles may be lost.

The gas chromatographic determination of ethanol is a well-established procedure. FID has been the universal detector, but TCD and MS have been used for some applications. Most analyses are performed using capillary columns with polar stationary phases. Historically, polar liquid phases were employed with packed columns. The phases currently on the market that can be used successfully for this purpose include Porapak Q/S, Carbowax 20 M, and Carbowax 20 M on Carbopack B.

Specimens may be subjected to distillation, protein precipitation, or solvent extraction for the separation of ethanol from the biological fluid. Most recently, the determination of ethanol and other volatiles has been accompanied by introducing the sample into the gas chromatograph either directly as a liquid or as a gas from the headspace.

16.8.2.2 Direct-Injection Technique

Machata (146) first used the direct injection technique to analyze ethanol in blood. He used GCFID with a packed column of polyethylene on kieselguhr to analyze 0.5 mL samples of blood and serum diluted with 0.2 mL of 0.25% acetone as an internal standard. When using the direct-injection technique, the liquid blood sample can be either injected directly or diluted prior to injection. The sample may also be extracted prior to the injection. Sample volume is typically 1–3 μL when using this procedure, and it is not uncommon for the needle to clog. Also, many nonvolatile components of the blood and other samples are injected into the injection port and consequently lodge onto the column requiring frequent maintenance. Many of the earlier methods used direct injection, and these have been thoroughly reviewed (147). The recommended procedure for direct injection is as follows:

1. Homogenize clotted or inhomogeneous samples.
2. Dilute samples (1–10) with aqueous internal standard to minimize maintenance.
3. Mix samples thoroughly.
4. Cap and analyze immediately (inject 1–3 μL).

A diluter/pipetter will give better reproducibility and allow more sample to be handled in a shorter amount of time than will diluting by hand. The method

described by Jain (148) is a typical example of a direct-injection procedure. This method simply mixes 0.5 mL of blood and 0.5 mL of internal standard solution (50 mg/100 mL isobutanol) with no extraction. Then 0.1–0.5 μL is injected onto a column of Carbowax 20 M on acid-washed 60/80-mesh Chromosorb W. The oven temperature is usually 100–130°C, with an injection port temperature of 160°C and an FID temperature of 200°C.

16.8.2.3 Static Headspace Procedure

According to Henry's law, the concentration of ethanol in the headspace of a blood sample in a closed vial is directly proportional to the concentration of ethanol in the blood solution when the system is in equilibrium. Thus the concentration of ethanol in the blood can be determined by measuring the peak area, or height, of a chromatographic peak resulting from a static headspace sample. The principles of Henry's law are described in Chapter 11 of this book.

Static headspace GC was originally developed for the determination of ethanol in blood (149), and today it is the method of choice for this application. Use of this technique offers distinct advantages over direct-injection methods. Most importantly is prevention of contamination of the column and syringe. The literature has numerous publications in this area describing various methods and studies of different factors affecting the determination of ethanol in blood. The most recent and perhaps state-of-the-art methods for the determination of ethanol involve automated headspace analysis using chromatographic systems that are capable of analyzing 30 samples in sequence. A recommended procedure for static headspace sampling is as follows:

1. Homogenize clotted or inhomogeneous samples.
2. Dilute samples (1–10) with aqueous internal standard (i.e., *n*-propanol) (may contain sodium chloride or sodium sulfate).
3. Mix samples thoroughly.
4. Cap and place in constant temperature bath.
5. Equilibrate and inject (25 μL–10 mL).

A diluter/pipetter will give better reproducibility and allow more samples to be handled in a shorter amount of time. The use of a high dilution factor and the addition of salt to the samples eliminates any differences between blood/air and water/air partition ratios, therefore allowing the use of aqueous standards for calibration. The addition of salt increases the volatilization of the ethanol and the internal standard, lowering the liquid/air partition ratio and improving sensitivity. This technique is called "salting out."

It should also be noted that at elevated temperatures ethanol can become oxidized to acetaldehyde, so the gas chromatographic procedure must separate ethanol from acetaldehyde. The author's laboratory historically used a 6-ft glass column (2 mm i.d.) packed with 5% Carbowax 20 M on Carbotrap B 60/80 mesh to perform blood alcohol analyses. When the oven temperature is held at

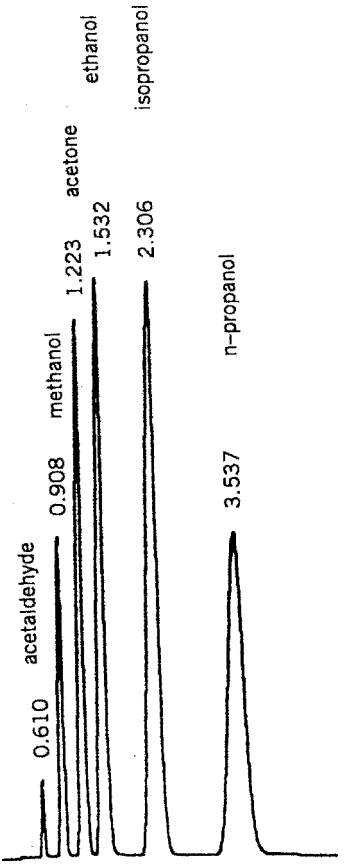


FIGURE 16.14 Gas chromatogram of a 1-mL headspace sample of a volatile mixture containing acetaldehyde, methanol, acetone, ethanol, isopropanol, and *n*-propanol. GCFID conditions: 6-ft glass column packed with 5% Carbowax 20 M on 60/80 Carbopack B; oven = 75°C; injection = 150°C, detector = 200°C; loop = 50°C. (Courtesy of New Jersey State Police.)

75°C isothermally, with a nitrogen flow of 30 mL/min, this column will separate ethanol from acetaldehyde with baseline resolution. Figure 16.14 shows the separation of a headspace injection of a mixture of volatiles including ethanol, acetaldehyde, and the internal standard, *n*-propanol. This separation is completed within 4 min. More recently however, we have modified the procedure to utilize a dual-column/dual-flame ionization detector system equipped with Restek Rtx BAC-1 and Rtx BAC-2 capillary columns and a headspace autosampler to produce two chromatograms with baseline resolution of all blood alcohol components in less than 3 minutes (Figure 16.15). Dubowski has detailed as an example, a headspace procedure that uses an internal standard (acetonitrile) in the *Manual for the Analysis of Ethanol in Biological Liquids* (150). The reader

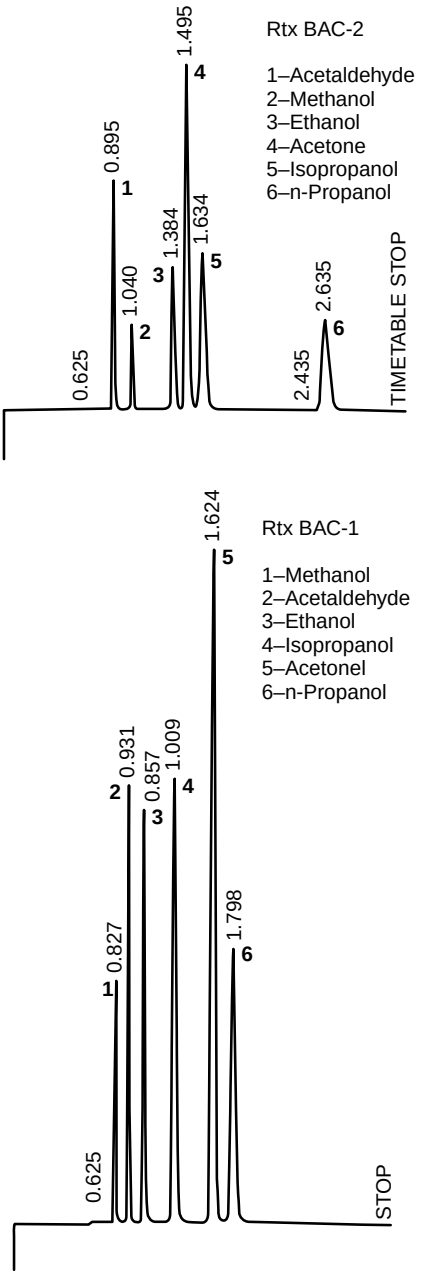


FIGURE 16.15 Gas chromatograms of a 1-mL headspace sample of a volatile mixture containing acetaldehyde, methanol, acetone, ethanol, isopropanol, and *n*-propanol. GCFID conditions: dual capillary columns RTx BAC-1 & RTx BAC-2 30-*m* × 0.33-mm-i.d. × 1.0-μm film; carrier gas helium = 7 mL/min; oven = 40°C; injection = 150°C, detector = 200°C; loop = 70°C. (Courtesy of New Jersey State Police.)

is referred to a review of static headspace applications for more information on this subject (151).

The practice of breath collection onto silica adsorbent for later analysis to compare to the results of an evidential breath-testing device (EBT) is currently being performed in some laboratories in the United States. The contents are emptied into a vial, diluted with an aqueous internal standard solution (*n*-propanol) and analyzed by headspace GC using procedures similar to those for blood alcohol analysis, but adjusted for sensitivity differences. Reanalysis of breath samples collected in this manner is not recommended, however, due to factors other than instrument performance, such as sample collection and operator errors.

16.8.2.4 Solid-Phase Microextraction

Solid-phase microextraction (SPME) is a relatively new solventless sample preparation technique that allows simultaneous sampling, extraction, preconcentration, and introduction of analytes from a sample matrix in a single procedure. The theory and methodology of this technique is described previously in this book (see Chapter 11). SPME has been applied to the analysis of several drugs of forensic toxicology interest as previously described and it has also been applied to the analysis of volatile compounds of forensic interest, including ethanol analysis. Automated headspace SPME has been used in conjunction with capillary GC in the analysis of samples for alcohol and other volatiles in blood and postmortem specimens, including vitreous humor (152). These methods use an internal standard such as isobutanol and show good linearity throughout the concentration range from 0.001 to 1.0 g/dL and detection limits of ethanol of approximately 0.0001 g/dL.

16.8.2.5 Miscellaneous

Gas chromatography is commonly used to perform the above procedures routinely in many forensic toxicology laboratories around the world. The precision of the blood alcohol procedure should show a coefficient of variation of 3% or less on replicate analyses, and should be accurate to 5% compared to primary standards. Samples should be run in duplicate and blanks should be run periodically to demonstrate that the system has no carryover. Quality assurance primary standards should be run periodically throughout the analyses to ensure linearity of the method. Various reference materials are available to prepare or use as standards and/or calibrators [e.g., the National Institute of Standards and Testing (NIST) material, SRM 1821 Ethanol, and the College of American Pathologists (CAP) alcohol reference materials, in addition to manufacturers' produced materials]. Documentation of the results of the standards, as well as instrument certification, maintenance history, and proficiency testing, is mandatory for good quality assurance. Chain of custody of samples and good recordkeeping are also mandatory in this area. An excellent resource for quality assurance is Forensic *Toxicology Laboratory Guidelines* (153), which has been approved by the American Academy of Forensic Sciences, Toxicology Section, and the Society of Forensic Toxicologists. Another excellent resource on blood alcohol analysis is Garriott's Medicolegal Aspects of Alcohol (154).

Forensic laboratories typically report BAC values differently than do clinical laboratories. Forensic BAC values are reported consistent with the state statute, such as grams of ethanol per 100 mL of blood (g/100 mL). Most clinical values are reported in mg% or mg/dL. In addition, most hospital laboratories report the results of serum alcohol, which is, on average, approximately 1.16 times higher than a whole blood reading.

PART 4 APPLICATIONS OF GAS CHROMATOGRAPHIC ANALYSIS OF TRACE EVIDENCE

16.9 DETECTION OF IGNITABLE LIQUID RESIDUES FROM FIRE DEBRIS WITH GAS CHROMATOGRAPHY

16.9.1 Introduction

Among the various responsibilities of the forensic science laboratory is the examination of physical evidence from the scenes of suspicious fires. Physical evidence at the scene of a suspicious fire may be placed in either of two broad categories: (1) residual materials of the type responsible for the initiation and acceleration of the fire and (2) physical evidence that may be primarily associated with one or more suspects in an incendiary fire (155). Examples of the latter are hair, paint, glass, blood, and fingerprints. With the exception of paint, GC is normally not utilized to analyze these latter examples, however GC has universally been the method of choice for analysis of ignitable liquid residues from fire debris.

Fires of a suspicious nature often involve the use of an accelerant, a material used by the arsonist to rapidly spread the fire. An accelerant may be a solid, liquid, or gaseous substance. The most commonly used accelerants are commercial flammable or combustible liquids, such as gasoline, kerosene, paint thinners, charcoal lighter fluids, alcohols, mineral spirits, and fuel oils. In the investigation of a suspicious fire, fire investigators first identify the location of the origin(s) of the fire and then the source of ignition. An obvious indication that a fire has been deliberately set is the severity of damage or unusual burn patterns indicative of the presence of an accelerant. Detection and identification of accelerants provides the investigator with scientific proof that the fire was incendiary and may help link the suspect to the crime.

Debris recovered from the fire scene is often wet and burned, and may consist of material such as wood, carpet, carpet padding, tile, and other synthetic materials, all of which can contribute interfering volatile pyrolysis products that can make the identification of the accelerants difficult. The loss of accelerants through adsorption into the debris, evaporation from the heat of the blaze, and the presence of water all contribute to make the identification of accelerants a challenging task. GC can be a powerful tool in the analysis to separate and identify the accelerant in the presence of these interferences. Fultz and DeHaan have written an excellent chapter on GC in arson and explosive analysis (156).

Several references are available for further reading on the general topic of fire investigations (157–160).

16.9.2 Collection and Packaging of Evidence

Because of the high vapor pressure of accelerants, the debris from the fire scene must be packaged in vapor-tight, clean, unused containers for transmittal to the laboratory. Improper packaging can lead to loss subsequent to collection of the samples. It is important for the laboratory to periodically check containers used by investigators for any contamination that could interfere with the chromatography. Clean, new, unused metal paint cans with friction lids are the most commonly used container to package fire debris. These cans come in a variety of sizes and are available lined to prevent rusting. Glass jars can also be used to package fire debris but have the disadvantage of being breakable. Liquid solvent or vapors in the sample may also destroy the rubber sealant in the lids.

Plastic bags have been investigated for packaging fire debris evidence. Polyethylene bags are permeable to hydrocarbon vapors and not suitable for arson evidence. Nylon film bags have been effective in retaining volatiles but can be difficult to seal and are easily punctured by sharp objects in the debris. These bags have gained some popularity, especially in Europe, but are not as popular in the United States.

16.9.3 Chromatographic Characterization of Ignitable Liquid Residues

Gas chromatography is the method of choice for the detection and characterization of accelerants from fire debris. Since petroleum products are by far the most common types of accelerants and because GC is used to characterize the type of accelerant, the forensic scientist must have a basic understanding of petroleum products and their manufacturing process.

The refining and manufacturing of petroleum products is basically a distillation procedure, with the commercial products being distributed accordingly. Fortunately for the analyst, generally it is only the flammable liquid products with high vapor pressure and low flash point that are used as accelerants with the liquid residues remaining among the fire debris. GC can easily separate these compounds, and, in fact, when using temperature programming of the gas chromatographic oven, the ignitable liquid residues can be placed into a relatively simple classification scheme based on overall chromatographic retention patterns and refining processes.

Table 16.11 shows the most recent classification scheme adapted by the American Society of Testing and Materials (ASTM) in 2001 (161). This classification is based on the elution of specific compounds within a retention time window defined by *n*-alkane carbon number. For example, for an accelerant to be classified in the light product range, the chromatogram must have the majority of the pattern occurring in the range from *n*-C4 to *n*-C9 with no major peak above

TABLE 16.11 Ignitable Liquid Classification Scheme[a]

Class	Light (C4–C9)	Medium (C8–C13)	Heavy (C9–C20)
Petroleum distillates	Petroleum ether, some cigarette lighter fluids[b], some camping fuels	Some charcoal starters, some paint thinners, some dry-cleaning solvents	Kerosene, diesel fuel, some jet fuels, some charcoal starters
Isoparaffinic products	Aviation gas specialty solvents	Some charcoal starters, some paint thinners, some copier toners	Commercial specialty solvents
Aromatic products	Some paint and varnish removers, some automotive parts cleaners, xylenes, toluene-based products	Some automotive parts cleaners, specialty cleaning solvents, some insecticide vehicles, fuel additives	Some insecticide vehicles, industrial cleaning solvents
Naphthenic paraffinic products	Cyclohexane-based solvents/products	Some charcoal starters, some insecticide vehicles, some lamp oils	Some insecticide vehicles, some lamp oils, industrial solvents
Normal alkane products	Solvents, pentane, hexane, heptane	Some candle oils, copier toners	Some candle oils, carbonless forms, copier toners
Dearomatized distillates	Some camping fuels	Come charcoal starters, some paint thinners	Some charcoal starters, odorless kerosenes
Oxygenated solvents	Alcohols, ketones, some lacquer thinners, fuel additives, surface preparation solvents	Some lacquer thinners, some industrial solvents, metal cleaners/gloss removers	
Others—miscellaneous	Single-component products, some blended products, some enamel reducers	Turpentine products, some blended products, various specialty products	Some blended products, various specialty products

[a] The products listed in this table in the various classes are illustrations of known commercial uses of ignitable liquids. These examples are not intended to be all-inclusive. Reference literature materials may be used to provide more specific examples of each classification. Gasoline (as listed here) includes all brands; Fresh gasoline is typically in the range C4–C12, including gasohol.

[b] As can be noted, there are products found in multiple classifications such as "charcoal starters"; therefore, many of the examples can be prefaced by the word "some," as in "some charcoal starters."

Source: From Reference 161.

n-C11. Examples of light product range are listed in Table 16.11. Figure 16.16 shows the chromatogram for a headspace sample of CAM 2 racing fuel, which would fall into the light product range. Similarly, for an ignitable liquid residue to be classified into the medium product range, the majority of the chromatogram pattern must occur within the range from *n*-C8 to *n*-C13. Figure 16.17 shows the chromatogram for a headspace sample of MAB paint thinner, which would fall

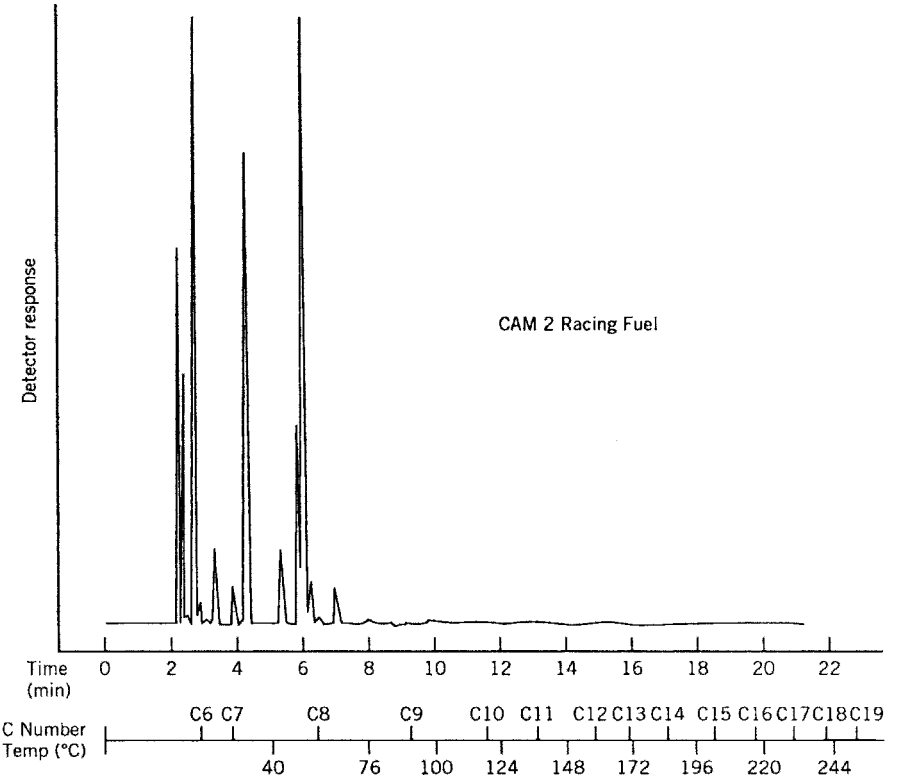


FIGURE 16.16 Gas chromatogram of a 1-mL headspace sample of CAM 2 racing gasoline (25-μL/qt can) heated for 20 min at 90°C. GCFID conditions: 30-m SPB-1 × 0.75-mm-i.d. × 1.0-μm film; 40°C (5 min) to 250°C at 12°C/min; injection = 260°C, detector = 280°C. (Courtesy of New Jersey State Police.)

into this medium product range. This classification scheme divides the ignitable liquid residues into a matrix depending on their chromatographic behavior and known commercial use.

Chromatographic characterization of ignitable liquids based on this classification requires enough column efficiency to separate the *n*-alkanes from butane up to tricosane (*n*-C23) in such a manner as to be able to evaluate unknown complex mixtures. Figure 16.18 shows the separation of a standard mixture of normal alkanes (from *n*-C6 to *n*-C28) that can be used for evaluation of ignitable liquids in this scheme. Under these conditions, sufficient separation and resolution is provided for identification of the products listed in this classification scheme. The chromatographic conditions for this evaluation usually require the use of a nonpolar liquid phase on which the elution order of most compounds can be directly related to boiling point. Methylsilicone liquid phases are the best stationary phases for this application and are widely used for ignitable liquid residue identification.

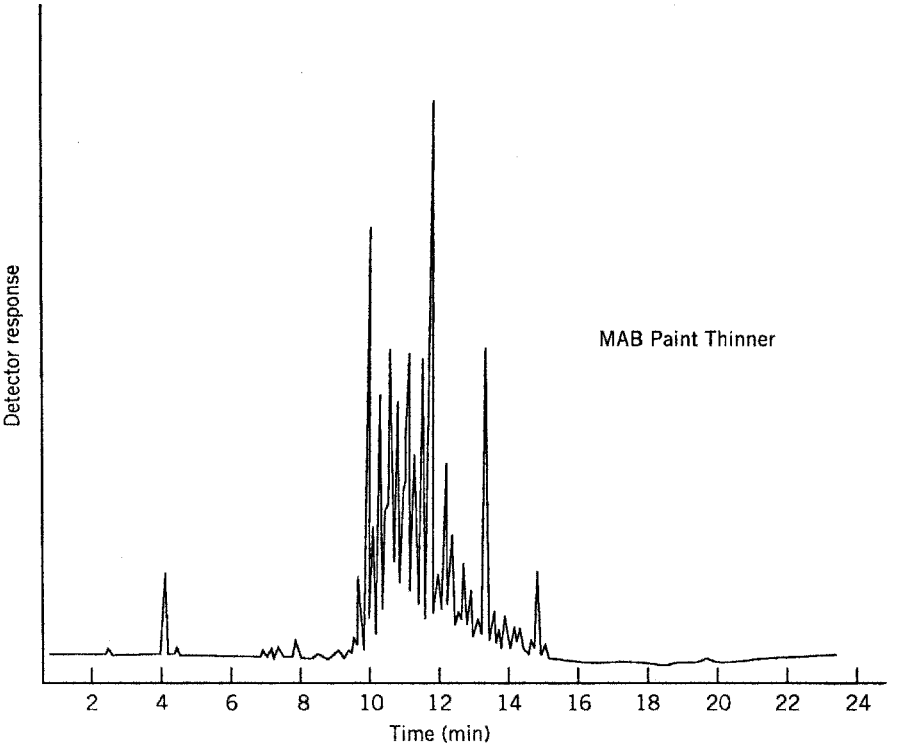


FIGURE 16.17 Gas chromatogram of a 1-mL headspace sample of MAB paint thinner (10-μL/qt can) heated for 20 min at 90°C. GCFID conditions: 30-m SPB-1 × 0.75-mm-i.d. × 1.0-μm film; 40°C (5 min) to 250°C at 12°C/min; injection = 260°C, detector = 280°C. (Courtesy of New Jersey State Police.)

No classification system is likely to describe all possible ignitable liquids. Gasoline produces chromatographic patterns distinctive enough to be placed into a separate class. Numerous commercial and industrial products are ignitable but fall into more than one category or do not fall into any of the above mentioned categories, other than "miscellaneous." Many of these are synthetic mixtures consisting of only a few compounds, rather than distillation fractions, and require multiple column analysis in order to achieve identification. GCMS would be needed when the gas chromatographic pattern is not sufficiently complex to identify a mixture of components.

Today, most laboratories use temperature programming and fused-silica capillary columns to separate the wide-boiling-point range of products that are used as accelerants. The particular column and chromatographic conditions used are not as important as long as the column can provide enough resolution to effectively separate and identify the complete range of accelerants. ASTM Standard Test Method E1387-01 (161) recommends that a test mixture of equal parts by weight

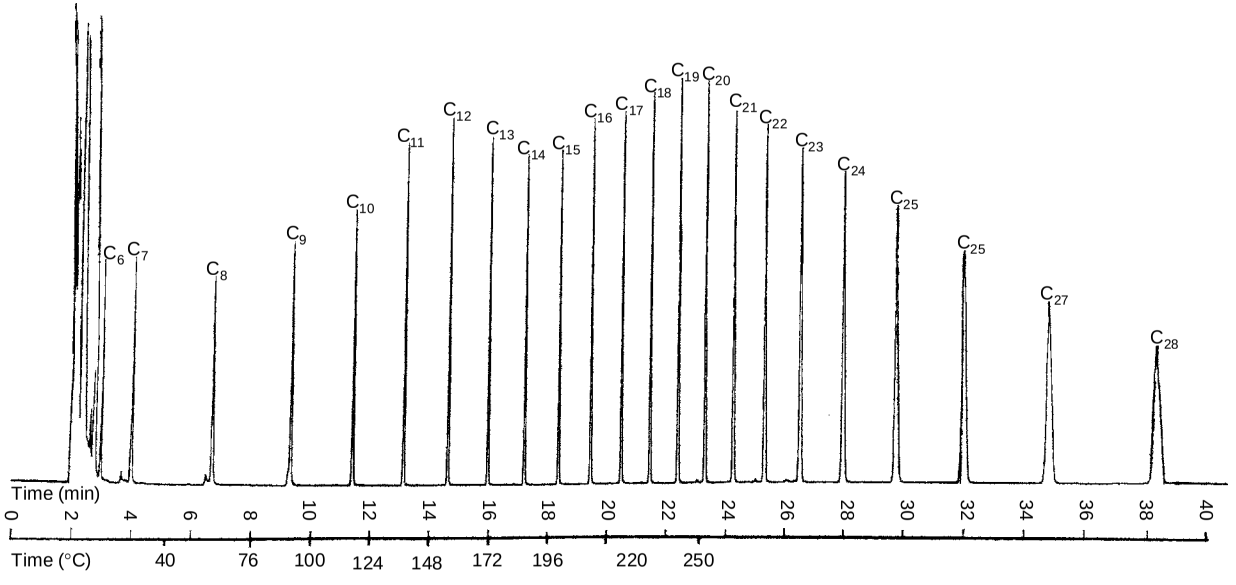


FIGURE 16.18 Separation of normal alkane standard on a SPB-1 methylsilicone glass capillary column (30-m × 0.75-mm-i.d. × 1.0-μm film); GCFID conditions: carrier gas helium = 6 mL/min; oven = 40°C (5 min) to 260°C at 12°C/min; injection = 260°C, detector = 280°C. (Courtesy of New Jersey State Police.)

of even-numbered normal alkanes ranging from *n*-octane through *n*-eicosane plus toluene, *p*-xylene, *o*-ethyltoluene, *m*-ethyltoluene, and 1,2,4-trimethylbenzene must be resolved for the column to be considered adequate for identification of ignitable liquid residues.

Because of the large number of components present in ignitable liquid products, they produce complex characteristic patterns that can be used to identify different accelerants by pattern-recognition techniques. Most laboratories build a library of chromatograms using the columns, conditions, and sample preparation technique that are most frequently used for casework. Chromatograms obtained by headspace sampling most often differ enough from chromatograms obtained by liquid injections to justify building separate chromatogram libraries. For this reason ASTM Standard E1387-01 recommends this in the procedure for analysis of ignitable liquid residues in extracts from fire debris samples by GC (161). That procedure states:

> 10.2.1 The essential requirement for making a classification using this procedure is the matching of the sample chromatogram with a known reference ignitable liquid chromatogram obtained under similar conditions, noting sufficient significant points of correlation or similarities. Make all comparisons using only good chromatograms. . ..
>
> 10.2.1.1 The use of externally generated libraries of chromatograms is not sufficient for identification of an ignitable liquid. Such libraries are intended to give guidance for selection of reference ignitable liquids.

The library of standards should also include chromatograms from common ignitable liquid products at various stages of evaporation. Ignitable liquid residues recovered from fire debris generally have been exposed to extreme heat and therefore have lost some or most of the volatile components through evaporation. This is commonly referred to as "weathering." Because of this evaporation, the patterns of the ignitable liquid products will differ depending on the extent of "weathering"; therefore they must become part of the library. Figures 16.19 and 16.20 show the headspace chromatograms of gasoline and 50% evaporated gasoline. The obvious loss of volatiles and pattern change can be seen easily in these chromatograms. It is recommended that the library contain several different standards of gasoline to include a range of weathering from unevaporated to at least 90% evaporated.

Pattern recognition and chromatogram interpretation can be a very difficult task in arson analysis. An excellent checklist in obtaining a chromatogram suitable for pattern recognition interpretation and steps used to interpret the chromatogram are given in Reference 156.

16.9.4 Sample Preparation

Several sample preparation techniques have been used to recover accelerants from fire debris. Not all are in use today, nor is there one technique that is universally applied to all types of flammable or combustible liquids. The sample

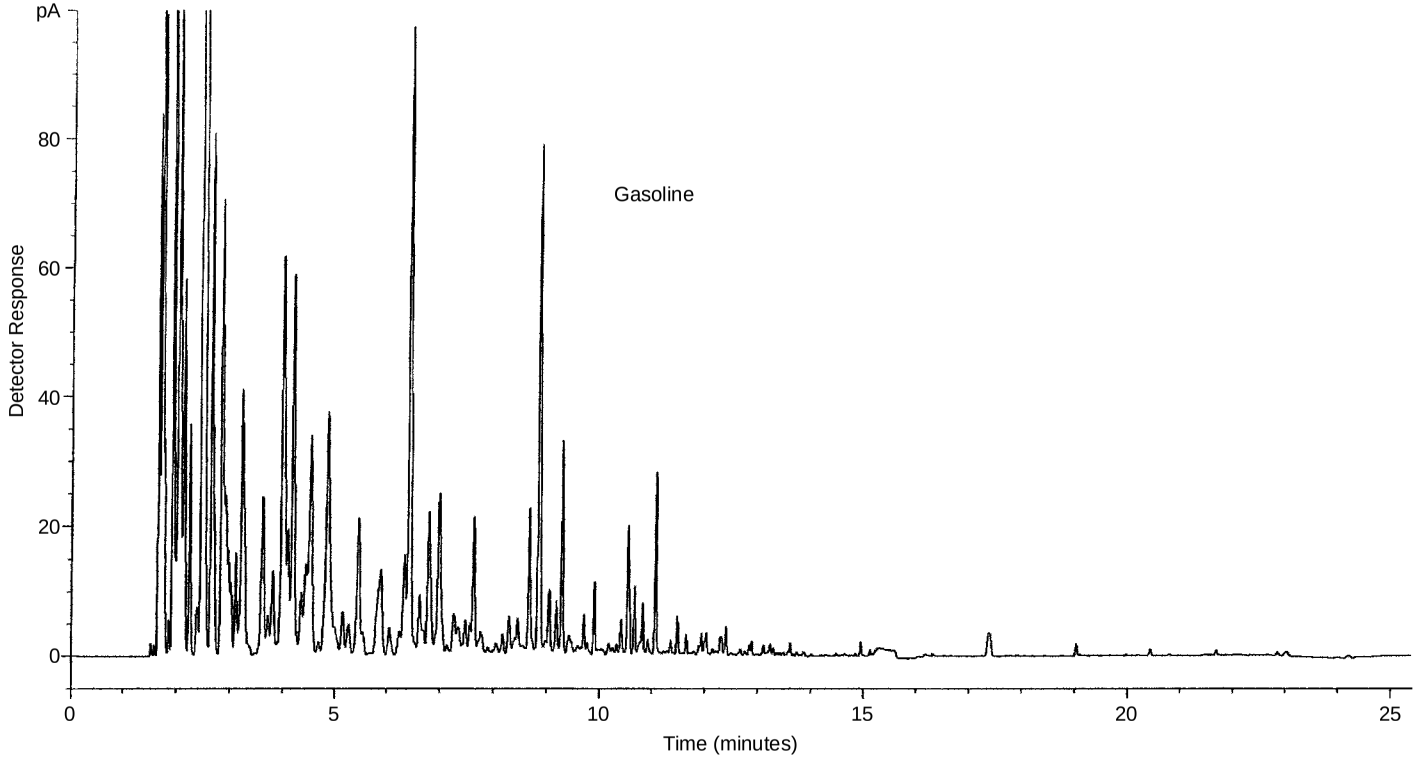


FIGURE 16.19 Gas chromatogram of a 1-mL headspace sample of gasoline (10-μL/qt can) heated for 20 min at 90°C. GCFID conditions: 30-m HP-1 × 0.53 mm-i.d. × 0.88-μm film; 40°C (5 min) to 250°C at 12°C/min; He carrier gas velocity = 35 cm/s; split ratio = 3:1; injection = 250°C, detector = 290°C. (Courtesy of New Jersey State Police.)

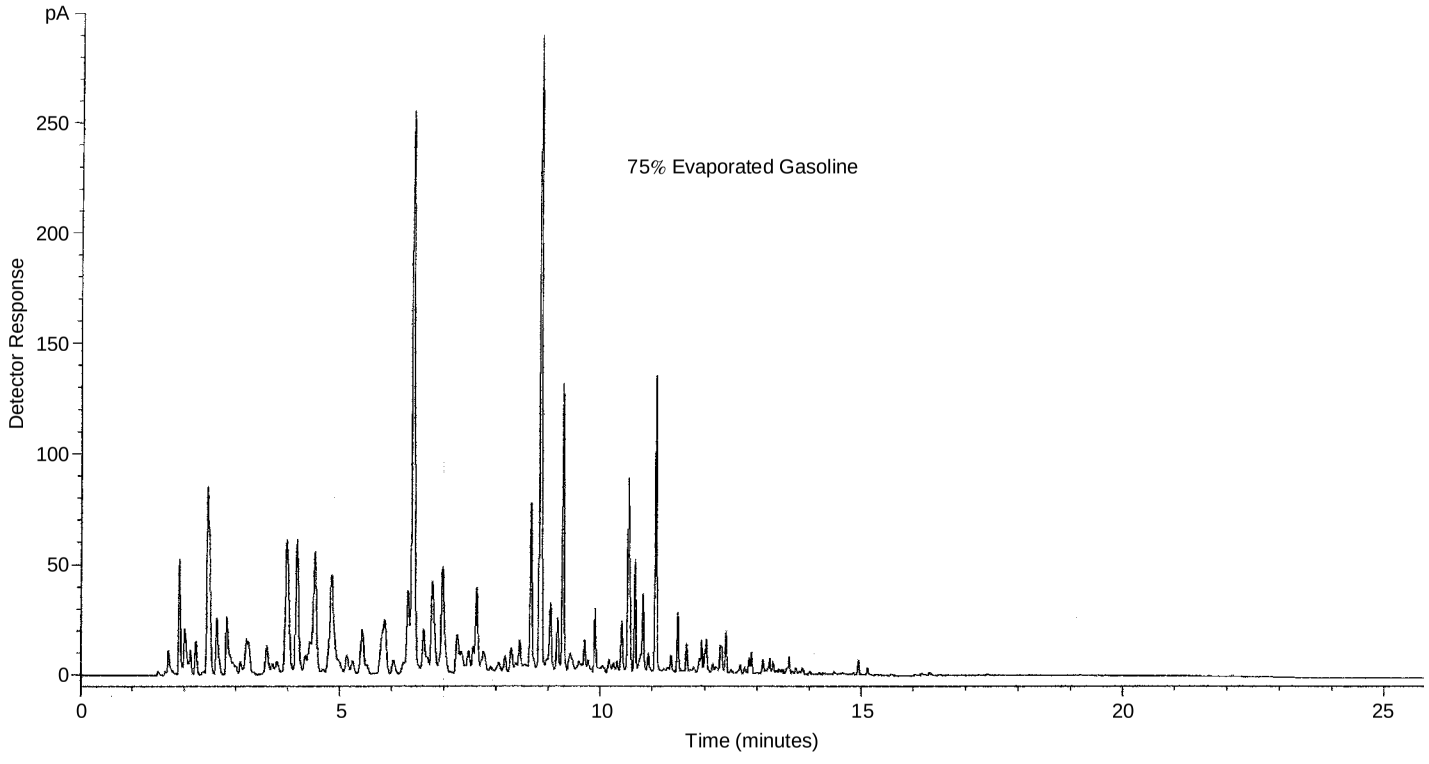


FIGURE 16.20 Gas chromatogram of a 1-mL headspace sample of 50% evaporated gasoline (10-μL/qt can) heated for 20 min at 90°C. GCFID conditions: 30-m HP-1 × 0.53-mm-i.d. × 0.88-μm film; 40°C (5 min) to 250°C at 12°C/min; He carrier gas velocity = 35 cm/s; split ratio = 3:1; injection = 250°C, detector = 290°C. (Courtesy of New Jersey State Police.)

preparation technique must be simple, efficient, rapid, free of contamination, and able to recover the sample in identifiable quantities. The analyst must be aware of the advantages and pitfalls of each method and must also recognize how the sample preparation technique will affect the chromatogram of the ignitable liquid residue. Caddy et al. (162) has reviewed the sample preparation methods used for the detection of ignitable liquid products in fire debris. Fultz and DeHaan (156) have previously proposed an analysis workflow that shows how these techniques fit into an overall analysis scheme for the identification of flammable and combustible liquids.

16.9.4.1 Distillation

Distillation methods were among the first techniques used to isolate petroleum products from fire debris samples. Distillation methods include simple distillation, steam distillation, and vacuum distillation. Ethylene glycol and vacuum distillations offer the highest recovery of petroleum products of the three distillation methods. These methods are time-consuming and cumbersome, however, and the complications involved in these methods make their usefulness for fire debris preparation uncommon. The procedure of separation and concentration of ignitable liquid residues in fire debris samples containing visible amounts of water has been outlined (163). These procedures are recommended only for samples that have a detectable odor of petroleum distillate at room temperatures. The advantage of this technique is that the distilled liquid can be harvested for further analysis, such as infrared analysis, and also for courtroom presentation, the odor being recognizable by the jury.

16.9.4.2 Solvent Extraction

Solvent extraction is one of the original techniques used to recover ignitable liquid residues from fire debris. The method is based on the solubility of the accelerant, generally hydrocarbons, in the extraction solvent and has the advantage of recovering nonvolatile materials that are not recoverable by other methods, generally because of their vapor pressure. The technique usually involves soaking the fire debris in a suitable solvent for a length of time, decanting the solvent, filtering, and then evaporating to a small concentrated volume for analysis. In this process, volatile accelerants may be lost and nonsoluble accelerants may not be recovered. In addition, a major disadvantage of solvent extraction is that contaminants and pyrolysis products may also be extracted, complicating the chromatogram and the analysis. One approach to clean up sample extracts was the removal of nonhydrocarbon materials by acid stripping (164,165). This method, using a mixture of phosphoric and sulfuric acids, does not appear to have achieved wide acceptance. A different approach in the cleanup was to use Florisil adsorbent, but this has not been routinely employed in the method (166).

Several extraction solvents have been studied. Table 16.12 lists some solvents that have been used in the recovery of fire debris. Practical guidelines have been published for the solvent extraction of flammable and combustible liquids from fire debris (177).

TABLE 16.12 Solvents Used in the Recovery of Petroleum Distillates from Fire Debris

Solvent	References
Carbon tetrachloride	167–169,171
Acetone	167
Carbon disulfide	167,168,173
Hexane	173,167,168
Ethyl ether	167,168,170,171
n-Pentane	167,168,172,174
n-Heptane	176
Petroleum ether	176
Dodecane	167,168
Hexadecane	167,168
Methylene chloride	167,168,175
Chloroform	167,168
Benzene	167,168

16.9.4.3 Static Headspace

Static headspace sampling (SHS) followed by GC has become one of the more common techniques for initial screening of fire debris evidence. The ease of sample preparation, the speed with which a large number of samples can be analyzed, and the versatility of handling different types of evidence, permit SHS followed by GC to be a very attractive and practical technique for accelerant detection. Although fast and effective with samples having high concentrations of neat or slightly volatile ignitable liquid residues, the SHS method suffers from effective recovery of the residue when very evaporated or heavy products are present. When there is a sufficient amount of any flammable or combustible liquid present in fire debris with enough vapor pressure to be present in the headspace of the container, however, SHS followed by GC will yield reproducible and meaningful results. Heating the sample container will increase the vapor pressure, yielding a higher concentration of accelerant vapors and improving sensitivity. The limit of detection is reported to be in the vicinity of not less than 5–10 μL of petroleum product in a gallon can (178). Many laboratories use a combination of SHS with another method, such as passive headspace, to recover heavier ignitable liquid residues more effectively.

As mentioned previously, the most common container for fire debris is an unused metal paint can. Normally a hole is punched in the lid of the can and sealed with tape before the can is sampled. Samples are typically heated in an oven or on a hot plate for 10–30 min at temperatures of 50–90°C. If enough accelerant is present to cause an odor, the analyst may elect to sample the container at room temperature. Heating samples that contain water above 90°C may cause the container to vent or burst. In addition, over heating the sample may cause pyrolysis of debris and complicate the interpretation of an already complex chromatogram. Typically, 0.5–3 mL of headspace vapor is removed from the

container and injected directly into the injection port of the gas chromatograph. The amount depends on the diameter and film thickness of the column. Procedures for sampling of headspace vapors from fire debris have been outlined (179).

The first preliminary work in which vapor examination of headspace samples was used for the analysis of traces of accelerants was reported by Midkiff and Washington in 1972 (180). Since then many studies have been performed in regard to heating conditions, container size, sample size, the effect of water, the degree of interference from pyrolysis products, and the chromatographic conditions, including the type and dimension of column.

Wide-bore columns have been shown to be advantageous in fire debris analysis, especially when headspace sampling is used (181). They offer high capacity, provide enough efficiency to separate the components of most accelerants and provide good differentiation between ignitable liquid product classes. Wide-bore columns also accommodate a variety of injection methods, some of which are not compatible with narrow-bore columns without accessories such as cryofocusing. The high capacity of wide-bore columns is a great advantage, especially when headspace sampling is used, since the analyst usually has little control of the amount of volatiles being sampled from fire debris using SHS. Narrow-bore columns can be easily overloaded in this case scenario.

Generally, SHS followed by GC is used as a screening technique, especially when an odor of an ignitable liquid residue is detected. The technique is particularly effective for accelerants with high vapor pressure or when single-component solvents are present. Chromatograms resulting from the headspace of accelerant vapors may differ from chromatograms of liquid flammables and the analyst must be wary of these differences in the interpretation of the chromatogram.

16.9.4.4 Passive Headspace

Passive diffusion of accelerant vapors onto an adsorbent placed inside the container of fire debris has gained wide acceptance in the United States because it is nonlaborious and takes little time to perform. Dietz (182) has reported a procedure that uses activated carbon-coated Teflon strips, similar to devices used in environmental monitoring badges, to recover as little as 0.2 μL of an equal mixture of gasoline, kerosene, and diesel fuel. Neuman (183) reported on the optimized method as well as the effects of time, temperature, strip size and sample concentration. The most recent recommended procedure heats the samples at approximately 60–80°C for 8–24 hs (184). The optimum adsorption time for maximum sensitivity will depend on the adsorption package, the sample itself, and the temperatures. The accelerants are desorbed with carbon disulfide, which can be concentrated to improve sensitivity and shows little response, but may cause a pressure disturbance to the FID. Another advantage to this technique is the fact that multiple analyses may be performed from one sample. The carbon strip can be easily cut into smaller pieces, placed into vials, and frozen for later analysis.

Earlier attempts at passive diffusion were not quite as successful. Variations of this technique included the use of Curie point pyrolysis wires coated with

finely divided activated charcoal and pyrolysis GC and charcoal-coated wires and elution with various solvents. These procedures did not gain general acceptance, and most laboratories now use passive headspace concentration with activated carbon strip approach followed by carbon disulfide elution.

Solid-phase microextraction (SPME) is well documented with respect to its convenience and applicability to sampling volatiles and as an extraction technique to detect ignitable liquid residues when coupled with GCMS (185–188). Nonetheless, fire debris analysts have yet to widely adopt SPME as a viable alternative to the activated charcoal passive headspace technique. SPME is a simple, solventless extraction procedure in which a phase-coated fused-silica fiber is exposed to the headspace above the fire debris packaged in a closed container. A drawback to the procedure requires a rubber sleeve septum be placed at the opening of the container for maximum recovery of analytes. The technique has been applied successfully for the detection of flammable and combustible liquid residues on human skin (189).

Standard guidelines have been proposed that recommend using a 100-μm polydimethylsiloxane-coated SPME fiber to detect C10–C25 compounds, while a 85-μm polyacrylate (PA) and a 75-μm Carboxen/PDMS have been shown to perform well for C1–C10 compounds (190). Exposure times for routine screening of samples are typically in the range of 5–15 mins, while optimum exposure time for maximum sensitivity will depend on the temperature and the concentration and composition of the volatile compounds present in the sample headspace. The fiber is normally desorbed into the injection port of the gas chromatograph for approximately 1.5–4 minutes at a minimum temperature of 200°C. The guidelines recommend the analysis of a fiber blank before each sample extraction. An ignitable liquid standards library should be maintained with neat and evaporated commonly ignitable liquids recovered from sample containers by this technique at various concentrations.

More recent work has reported the expansion of SPME sampling from the customary thermal desorption mode to solvent-based analyte desorption for the analysis of ignitable residues (191). This method used SPME extraction fibers that were desorbed by 30 μL of nonaqueous solvent to yield a solution amenable to conventional GCMS analysis with standard autosampler apparatus. This approach retained the advantages of convenience and sampling time associated with thermal desorption while simultaneously improving the flexibility and throughput of the method. Based on sampling results for three ignitable liquids (gasoline, kerosene, and diesel fuel) in direct comparisons with the widely used activated-charcoal strip (ACS) method this methodology appears to be a viable alternative to the routinely used ACS method.

16.9.4.5 Dynamic Headspace

Dynamic headspace sampling (DHS) is a nonequilibrium process in which air or an inert gas such as nitrogen is passed over the sample (in the case of a solid) or through the sample (in case of a liquid). In the case of a liquid sample, this is more commonly referred to as the *purge-and-trap* technique, which is used widely in the environmental field.

DHS methods are more sensitive than SHS methods and are not as cumbersome or time-consuming as distillation methods. However, like other headspace techniques, DHS procedures are best suited for light and medium ignitable liquid residues and do not give good recoveries of residues in the high-boiling class, such as diesel fuel.

Samples collected on adsorbents can be desorbed by heat (thermal desorption) or by solvent extraction. Thermal desorption of samples from charcoal is not efficient however, because of the high temperature needed (950°C) to remove hydrocarbons from the charcoal (192). For this reason, most ACS passive headspace procedures use carbon disulfide to extract the adsorbed liquid residues. In 1967 Jennings and Nursten (193) reported concentrating analytes from a large volume of aqueous solution using activated charcoal as the adsorbent and extracting with carbon disulfide. Since then many adaptations of this method have been used to detect accelerants in fire debris, but currently dynamic headspace methods are seldom used because of the inconvenience of sampling and possible contamination issues with equipment.

Thermal desorption from porous polymers is theoretically the most sensitive method for detecting ignitable liquid residues from fire debris, since the entire sample that has been trapped is injected into the gas chromatograph, rather than a portion of diluted sample. Since desorption efficiency of charcoal is poor, thermal desorption methods have used other sorbents. Tenax GC, a porous polymer capable of trapping a wide range of hydrocarbons, has been particularly useful to concentrate ignitable liquid residues from fire debris (194). DHS methods using this type of sorbent have shown increased sensitivity over static headspace methods of several orders of magnitude. One of the major advantages of Tenax GC over other adsorbents such as charcoal is the fact that it does not adsorb water. The use of Tenax GC and DHS has been successfully used to recover charcoal lighter fluid, gasoline, and kerosene at scenes up to 15 h after the fire has been extinguished.

Andrasko (195) compared Porapak Q, Tenax GC, and Chromosorb 102 for effectiveness of trapping hydrocarbon vapors from fire debris. The study found that although Porapak Q and Chromsorb 102 seemed to trap the vapors more strongly, Tenax GC had the best desorption efficiency and therefore was more suitable for analysis of fire debris.

Practical guidelines for using dynamic headspace concentration methods have been outlined (196).

16.9.4.6 Detection

Until the last few years (as of 2003), the most popular detector used in the gas chromatographic detection of ignitable liquid residues in fire debris has been the FID. It offers adequate sensitivity and because of the complex chromatogram that is generated with FID (pattern recognition), it has been used by most laboratories for class identification of ignitable liquid products. The photoionization detector (PID) and the thermionic ionization detector (TID) have both found applications in the analysis of ignitable liquid residues however, both are seldom used in this application.

New methodologies and the use of selected-ion monitoring (SIM) have made GCMS the detector of choice. GCMS has been used in the past for the detection and identification of single components, such as solvents, or ignitable liquid products with few components. Forensic scientists recognized the value of the mass spectrometer for identification of compounds in arson debris as early as 1976 (197). It had not been used routinely in the crime laboratory, however, until recently.

Since the early 1990s the cost of mass spectrometers has dropped into a range that has made them affordable to the local forensic laboratory. Until relatively recently even those laboratories that operated mass spectrometers lacked the data-handling capabilities to analyze the number of components in a complex chromatogram, such as those generated from an ignitable liquid residue in fire debris. Today, mass spectrometers are equipped with low-cost data-handling systems and software, allowing for the analysis of the complex mixtures from residue matrices and powerful data manipulation to facilitate data interpretation. Because it is not feasible to identify each peak from the chromatogram, especially when using high-resolution capillary columns, most applications have concentrated on the use of mass chromatography and the use of selected-ion monitoring to identify and differentiate ignitable liquid residues from background and pyrolysis products.

Smith (198) reported a GCMS method using mass chromatography for the identification of ignitable liquid residue products. This method used several characteristic ions for each of the major classes of compounds present in product. This and similar GCMS methods have lead to a standard method using extracted ion profiles that has been generally accepted by the forensic community (199). Using this method, the sample is analyzed with a gas chromatograph interfaced with a mass spectrometer and a data system capable of storing and manipulating chromatographic and mass spectral data. Postrun data analysis generates extracted ion profiles (mass chromatograms) characteristic of the chemical compound types commonly found in ignitable liquids. The major ions present in mass spectra of common flammable and combustible liquids are listed in Table 16.13. Additionally, specific chemical components (target compounds) may be identified by their mass spectra and retention times. As an example, the target compounds for gasoline are listed in Table 16.14. Semiquantitative determination of the target compounds that are identified by mass spectra and retention time may then be used to develop target compound chromatograms (TCCs). The total-ion chromatogram (TIC), or extracted ion profiles (EIP) for the alkane, alkene, alcohol, aromatic, cycloalkane, ester, ketone and polynuclear aromatic compound types, or TCC, or combination thereof, are then evaluated by visual pattern matching against known reference ignitable liquids. Finally, the ignitable liquids may then be grouped into one of eight major petroleum classifications or one miscellaneous class, as previously described (see Table 16.11). Figure 16.21 shows the mass chromatograms that result from an evaporated gasoline sample.

Mass chromatography is not needed for every fire debris sample analyzed in the forensic science laboratory; however, for those samples that are inconclusive

TABLE 16.13 Major Ions Present in Mass Spectra of Common Flammable and Combustible Liquids

Compound Type	Characteristic Ions (m/z)
Alkane	43,57,71,85
Cycloalkane and alkene	55,69
n-Alkylcyclohexanes	82,83
Aromatic alkylbenzenes	91,105,119; 92,106,120
Indanes	117,118,131,132
Alkylnapthalenes (condensed ring aromatics)	128,142,156,178
Alkylstyrenes	104,117,118,132,146
Alkylanthracenes	154,168,182,196
Alkylbiphenyls/acenaphthenes	154,168,182,196
Monoterpenes	93,136
Ketones	43,58,72,86
Alcohols	31,45

Source: From References 198 and 199.

TABLE 16.14 Gasoline Target Compounds

Compound		CAS[a] Number
1	1,3,5-Trimethylbenzene	108-67-8
2	1,2,4-Trimethylbenzene	95-36-3
3	1,2,3-Trimethylbenzene	526-73-8
4	Indane	496-11-8
5	1,2,4,5,-Tetramethylbenzene	95-93-2
6	1,2,3,5,-Tetramethylbenzene	527-53-7
7	5-Methylindane	874-35-1
8	4-Methylindane	824-22-6
9	Dodecane	112-40-3
10	4,7-Dimethylindane	6682-71-9
11	2-Methylnaphthalene	91-57-6
12	1-1-Methylnapthalene	90-12-0
13	Ethylnaphthalenes (mixed)	1127-76-0
14	1,3-Dimethylnaphthalene	575-41-7
15	2,3-Dimethylnaphthalene	581-40-8

[a] *Chemical Abstracts* Services.

Source: From Reference 199.

or when accelerant identification cannot be made from the GCFID pattern, GCMS must be used. For a comprehensive discussion on mass chromatography, the reader is referred to Chapter 7 in this book.

GC/Fourier transform infrared spectroscopy (GC/FTIR) has been used for the identification of ignitable liquid residues in fire debris samples (200a). Various

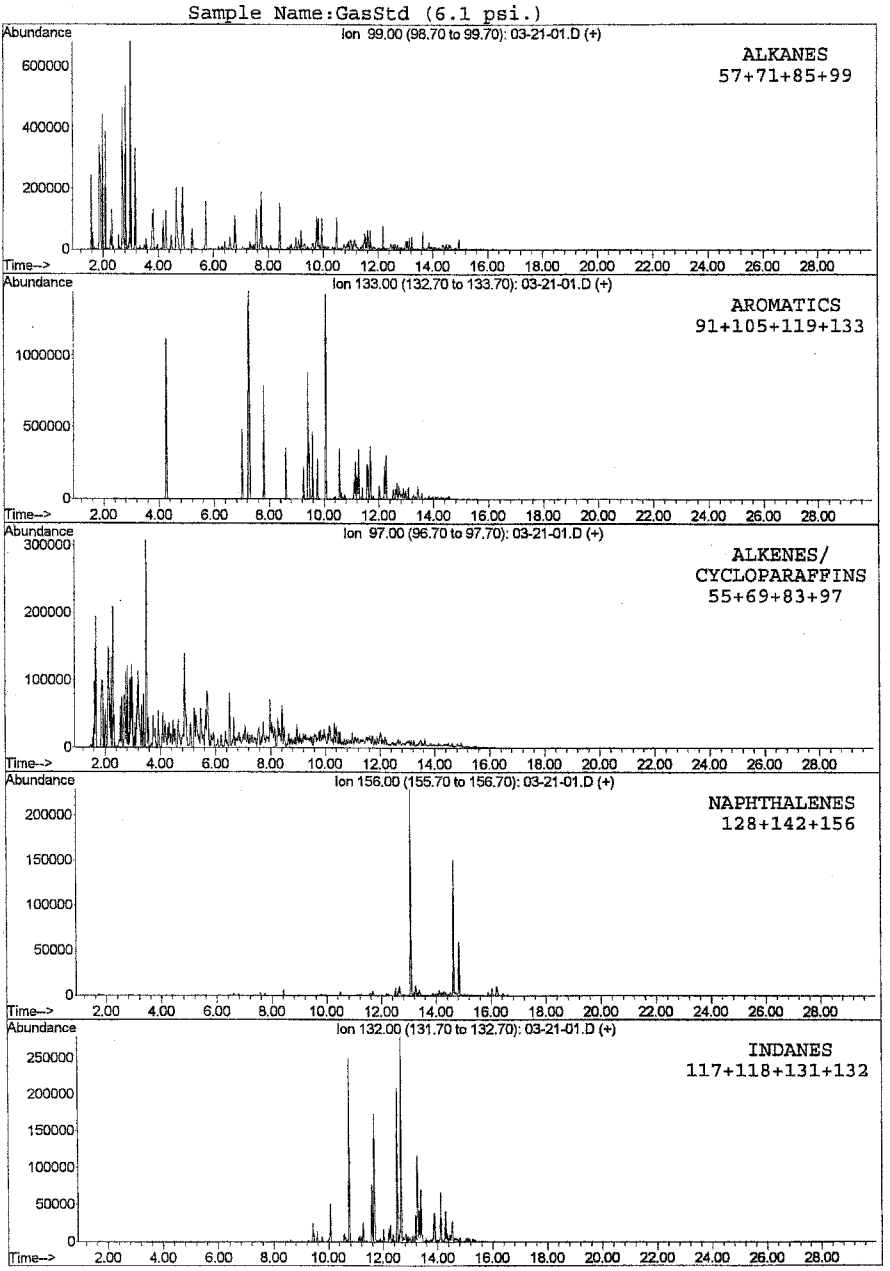


FIGURE 16.21 Family mass chromatogram from evaporated gasoline (courtesy of New Jersey State Police).

accelerants are analyzed to determine whether characteristic IR absorption bands could be used to identify each type of product. In a comparison to FID and MS detectors the FTIR is the least sensitive and does not discriminate accelerants from common pyrolysis products as well as GCMS.

The application of comprehensive two-dimensional GC (GC/GC) for the forensic analysis of ignitable liquid residues in fire debris has been reported (200b). GC/GC is a new, high resolution, multidimensional gas chromatographic method in which each component of a complex mixture is subjected to two independent chromatographic separations. The high resolving power of GC/GC can separate hundreds of chemical components from a complex fire debris extract. The GC/GC chromatogram is a multicolor plot of two-dimensional retention time and detector signal intensity that is well suited for rapid identification and fingerprinting of ignitable liquids. GC/GC can be used to identify and classify ignitable liquids, detect minor differences between similar ignitable liquids, track the chemical changes associated with weathering, characterize the chemical composition of fire debris pyrolysates, and detect weathered ignitable liquids against a background of fire debris pyrolysates. However, since this method is relatively new and highly sophisticated, it is not routinely used in the crime laboratory.

16.9.5 Comparison of Gasoline Samples

The forensic scientist is occasionally asked to compare brands of gasoline for the determination of common source or for the identification of the brand used in the arson. Unfortunately, brand identification is extremely difficult and, depending on the sample, it may even be impossible (155). The main reasons for this difficulty are the marketing practices of the refinery industry and the changes the product undergoes in storage.

Despite these limitations there has been some published work on gasoline comparison as to source. Historically, this type of work has been most difficult because of the volatile nature of the samples. In the past the comparisons have depended on the degree of weathering of the lower-boiling hydrocarbons in gasoline, the use of lead and lead isotopes (201), the use of other additives such as methyl-tertiary-butyl ether, and major refining and formulation changes. Mann (202) reported a comparison method to differentiate between unevaporated liquid gasoline samples, which involved measuring the quantitative differences of compounds eluting in the *n*-butane to *n*-octane region of the chromatogram when using a capillary column yielding baseline resolution of the compounds. Mann (203a) further extended this work to fire debris samples. In both reports the method was able to differentiate between samples not having a common source, but a conclusive determination of common origin was not possible.

These approaches are limited and many times difficult to demonstrate and apply. A more recent method has described a technique for estimating the age of regular/mid-grade gasolines using GC (203b). This method is based on the progressive enhancement of the aromatics and the reduction of the normal alkanes (paraffins) in the production of gasoline and using and index that reflects these

changes. The method can be successfully applied to liquid gasoline samples where the evaporation is less than 50%.

16.10 EXPLOSIVES ANALYSIS WITH GAS CHROMATOGRAPHY

16.10.1 Introduction

The forensic identification of bulk explosives and postexplosion residues is important in bombing investigations. The information can be used to determine the type of explosive, to link the suspect to the explosive, and ultimately to provide evidence in court. Many analytical techniques have been applied to the identification of explosives and explosive residues and several texts have been written on the subject (204–207).

The small amount of residue recovered and the interfering compounds present in the postblast debris complicate the unambiguous identification of an explosive at the scene. GC can be a valuable tool in the separation of the explosive from the interfering substance and when coupled with a specific and sensitive detector can give the unambiguous identification desired. GC is widely used for the analysis of explosives and explosive residues, despite the fact that many explosive compounds are inherently thermally unstable.

Basically there are two types of explosives, high- and low-order explosives, which are primarily differentiated by the burning rate and the manner of initiation. Low-order explosives burn more slowly and are usually initiated with a burning fuse or other heat source. High-order explosives are initiated by shock, usually through a booster charge or another explosive, such as a blasting cap. Examples of low-order explosives are black powder, pyrodex, single- and double-based smokeless powders, and flash powders. Analytical methods for examining this type of explosive include techniques other than GC, such as microchemical spot tests and thin-layer chromatography.

High-order explosives generally fall into three categories: commercial, military, or improvised, depending on the intended use and manufacturing process. Table 16.15 lists some explosives that have been analyzed by GC. Gas chromatographic methods for the examination of a variety of explosives have been reported (208). Prior to the introduction of fused-silica capillary columns, gas chromatographic analysis was limited to bulk explosives of nitroaromatics, because of the thermal lability of these compounds. The polarity of this class of compounds also rendered analysis on packed columns difficult because of irreversible adsorption on most liquid phases. Glass columns, on-column injection, and low-temperatures were common techniques used to prevent decomposition.

Trace analysis of explosives in postblast debris samples requires a detector considerably more sensitive than an FID. ECD, nitric oxide detection (TEA), and GCMS have all been used for the chromatographic detection of explosives (209). Gas chromatographic analysis with specific detection is normally used for screening purposes. Other methods, such as TLC, microscopy, X-ray diffraction (XRD), and SEM, are often used to confirm the presence of trace

TABLE 16.15 Commercial and Military Explosives Analyzed by GC

Name(s)	Abbreviation
Dinitrotoluene isomers	DNT
Trinitroluene	TNT
Ethylene glycol dinitrate	EGDN
Nitroglycerin (glycerol trinitrate)	NG
Nitrocellulose (cellulose nitrate)	NC
Isosorbide dinitrate	ISDN
Pentaerythritol tetranitrate	PETN
1,3,5,7-Tetranitro-1,3,5, 7-tetrazacyclooctane	HMX
1,3,5-Trinitro-1,3,5-triazacyclohexane	RDX
2,4,6-*N*-Tetranitro-*N*-methylaniline	Tetryl

amounts of explosives. A review of the analysis of explosive residues using a variety of analytical methods has been published (210).

16.10.2 Electron-Capture Detection of Explosives

The electron-capture detector (ECD) was the first detector available that had the necessary selectivity and sensitivity to detect trace amounts of explosives (211). Douse used silica capillary columns coated with OV-101 to separate picogram quantities of explosives (212). Jane et al. used this GCECD method to identify nitroglycerin in gunshot residue (213). NG, TNT, and RDX in the low-nanogram range were detected in handswab extracts using a 12-m $\times$ 0.25-mm BP-1 fused-silica capillary column and a ^{63}Ni ECD (214). Twibell et al. (215) also analyzed organic explosives from handswabs, and their work indicated that GC/ECD was the most sensitive technique for this kind of work.

In 1982, Yip used short, mixed liquid phases and combined packed capillary columns to separate EGDN, EGMN (ethylene glycol mononitrate), and NG at levels of 10^{-12} g/mL (216). A method has been reported using a packed column with dual detection using an ECD and photoionization detector (PID) and response ratios for the identification of TNT, RDX, Tetryl, and NG (217).

Belkin et al. detected ppb levels of TNT-type explosives by capillary-column GCECD (218). Hobbs and Conde developed a headspace technique utilizing capillary column GCECD to detect vapors of several types of explosives (219).

16.10.3 Thermal Energy Analyzers

The thermal energy analyzer (TEA) is a specific detector for the measurement of *N*-nitroso compounds and also responds to nitrosoamines, musk oils, foam blowing agents, and others. The TEA is based on chemiluminescence and was used to detect explosives as early as 1978 (220). The principle of the detector involves

the pyrolysis of *N*-nitroso compounds to release NO_2 that emits a characteristic infrared chemiluminescence, which is detected by a photomultiplier tube. The TEA is linear over six orders of magnitude and sensitive to *N*-nitroso compounds in the picogram range. Fine et al. used the TEA to examine postblast debris and handswabs from volunteers handling gelatin dynamite or the military explosive, C4 (221). On-column injection into a 30-m × 0.32-mm DB-5 fused-silica capillary column with TEA detection was used to detect picogram amounts of NG, PETN, ISDN, EGDN, 2,4-DNT, TNT, RDX, and Tetryl (222).

For the analysis of explosives, TEA has been found to be superior in sensitivity and selectivity over FID, ECD, and thermionic specific detector (TSD). Douse reported the TEA detection of explosives from handswabs in the low-picogram range using heated splitless injection with fused-silica capillary columns (223). Several modifications have been made to improve the sensitivity of the TEA detector. One variation involved the development of a method to trap high-performance liquid chromatographic eluent from a microcolumn and inject the eluent directly onto a gas chromatographic retention gap of unmodified silica, eliminating the need for evaporation and concentration prior to the analysis by GCTEA (224). Kolla used GCTEA to analyze trace amounts of explosives that were extracted from debris using solid-phase extraction (225). A portable explosives detector, the EGIS, combines an air sampler and a high-speed GC analysis with the TEA to give in seconds results that are comparable to those obtained with the laboratory configuration (226).

16.10.4 Gas Chromatography/Mass Spectrometry

Gas chromatography/mass spectrometry (GCMS) has been used only to a limited extent for the analysis of explosives due to the thermal instability of the explosives in the heated gas chromatograph (see Chapter 7). However, it is a powerful tool for the detection of low levels of organic explosives in postexplosion debris and for identification of nitroesters and nitroaromatics. Fused-silica capillary columns have increased the sensitivity of the technique and chemical ionization (CI), a soft ionization technique that yields molecular weight information, has helped renew interest in the method. The combination of CI and EI mass spectra complement each other and provide a reliable identification of explosive compounds. Negative-ion mass spectrometry (NIMS), has also been used for explosive analysis, which has led to increased sensitivity and selectivity. Using this technique with a fused-silica capillary column, TNT was analyzed with a detection limit of 50 pg (227). Pate and Mach reported the separation and identification of EGDN, NG, 2–4-DNT, and TNT at the nanogram level on glass packed columns using CIMS (228). Tamiri and Zitrin have described the application of GCMS to confirm the results of a TLC analysis of postblast explosive residues (229).

GCMS has been used for the analysis of smokeless gunpowders. Thirty-three commercial smokeless powders were compared using GCMS with EI and CI (methane) (230). The same investigators unsuccessfully attempted to use this

procedure to test for the presence of gunshot residue (GSR) found on hands by measuring either traces of the original volatilizable organic constituents present in the smokeless powder or characteristic organic compounds formed during the firing process (231). GC/EIMS was later used to analyze recovered unreacted GSR particles from double-base powder (232). Martz et al. developed a library of reloader smokeless powders based on electron impact mass spectral data of the volatile components of the smokeless powders (233). Selavka et al. replaced high-performance liquid chromatographic methods of analysis for smokeless powder additives by GCFID (234). GCFID was also used to characterize smokeless powder flakes from fired cartridge cases and from discharge patterns on clothing (235). Furton et al. reported on a critical evaluation of SPME for the rapid analysis of high explosives in water and postexplosion residues combined with separation methods including GC, HPLC, and micellular electrokinetic capillary chromatography (MECC) (236).

For further review of the application of GCMS and MS/MS to the forensic identification of explosives refer to the studies by Yinon (237, 238).

16.11 FORENSIC SCIENCE APPLICATIONS OF PYROLYSIS GAS CHROMATOGRAPHY

16.11.1 Introduction

Pyrolysis consists of the thermal transformation of a compound into another compound or compounds, usually in the absence of air. The usefulness of pyrolysis is the technique's ability to convert a nonvolatile organic material into a number of volatile organic compounds that can be separated by GC, identified, and related to the chemical composition of the original material.

The combination of pyrolysis and GC (PGC) is a powerful and sensitive method for discriminating certain types of physical evidence. PGC has been applied to a wide variety of sample types, but by far the major application of PGC has been in polymer analysis. In the crime laboratory, PGC provides one of the most discriminating tests for forensic paint comparisons.

Dr. Paul Kirk and his coworkers were first to apply and develop many of the applications and techniques of PGC to various types of physical evidence (239), such as commercial plastics, paints, and drugs. The application of PGC to forensic samples has been reviewed by Wheals (240) in 1981, by Saferstein (241) in 1985, and by Blackledge (242) in 1992. A bibliography of analytical pyrolysis, including forensic applications, was published by Wampler (243) in 1989.

16.11.2 Pyrolysis Gas Chromatographic Methods

PGC can be classified into two distinct types, depending on the method in which heat is applied to the sample: static-mode (furnace) reactors and dynamic (filament, pulse-mode) reactors. Furnace-type pyrolysis systems are seldom used in forensic laboratories. The most common pyrolysis systems used are the Curie

point (inductive heating) and the ribbon or filament-type pyrolyzers (resistive heating). A brief description of each pyrolysis system is provided below. For more detailed information the reader is referred to other texts on the subject (241,244).

16.11.2.1 Curie Point Pyrolysis (Inductive Heating)

Curie point pyrolysis uses a high-frequency induction coil to heat a ferromagnetic wire containing the sample to the wire's Curie point (the temperature at which the ferromagnetic wire becomes paramagnetic). The sample is centered in a glass or quartz tube, connected to the inlet of the gas chromatograph in a position to be in the flow of the carrier gas. Proper control of the pyrolysis conditions (temperature and flow), including wire composition, is required to obtain repeatable pyrolysis data. The major advantage of the use of a pulse-mode heating arrangement is that the sample is heated quickly and the products are removed from the hot zone before any significant secondary reactions occur.

Curie point pyrolysis is widely used by forensic laboratories in Europe and Asia. Forensic scientists in the United States seldom use it, basically because of the lack of marketing and available service to these units.

16.11.2.2 Filament and Ribbon Pyrolysis (Resistive Heating)

In this type of system a resistively heated platinum or nichrome wire coil or ribbon is used to rapidly heat the sample. The wire is continuously swept with carrier gas, whereupon the pyrolysis vapors are transported into the chromatographic column. Heating times are relatively large (up to 20 s) for this system, which may lead to nonrepeatable pyrograms and secondary reactions. The pyrolysis conditions, sample size, and location must be carefully controlled to obtain repeatable data. Two possible heating modes are available for this system: pulse mode or programmed mode. For most forensic applications the pulse mode has been used.

Various commercial pyrolysis systems are available that offer filament or ribbon resistive heating. Forensic laboratories in the United States almost exclusively use various models of the Pyroprobe (Chemical Data Systems, Oxford, PA). The Pyroprobe systems use either a self-sensing resistivity platinum wire coil or ribbon to heat the sample. The coil probe is used for solid samples, viscous liquids, and semisolids that are not soluble in a volatile solvent. Samples are normally placed in a quartz boat or between quartz wool plugs in a quartz tube. The sample should be centered and placed in nearly the exact spot of the tube or boat for good repeatability. Care must also be taken not to contaminate either the sample or holder when preparing for analysis. Autosampling is now available to increase the throughput and allow the scientist to gather more data on a sample in a shorter period of time.

16.11.3 Applications

16.11.3.1 Paint

Paint is one of the more common types of physical evidence that is submitted to the crime laboratory. Paint evidence may originate from a number of different

sources, such as tools, household items (windows and doors), and automobiles. The forensic examination of paint evidence involves a scheme of analysis with a variety of analytical techniques, such as microscopy, solvent tests, scanning electronmicroscopy with energy-dispersive X-ray spectrometry (SEM-EDXS), infrared spectroscopy (IR), inductively coupled plasma (ICP), X-ray diffraction (XRD), and PGC.

PGC is one of the more valuable techniques available to the forensic scientist for examining paint specimens. Application of forensic PGC of paint samples with a wide range of pyrolysis operating conditions has been reported over the years. Because of this variation in conditions and the difficulties in reproducibility, forensic application of PGC has suffered from a lack of standardization. Despite these difficulties, PGC has been shown to be sufficiently characteristic and reproducible to differentiate different manufacturers of similar paints (245–247).

The discrimination power of PGC in a paint comparison is markedly linked to the gas chromatographic stationary phase employed (241). However, in an effort to increase the reproducibility most laboratories have adopted a single (15–30-m) fused-silica capillary column with a nonpolar (methylsilicone) or medium-polar (phenylmethylsilicone) stationary phase. Over the years many different types of phases of various polarities have been evaluated for their discriminating power of paint samples by PGC, including, but not limited to, Carbowax 20 M and Porapak Q.

While capillary columns have improved the resolution of pyrolyzate compounds, the type of stationary phase is still important in the discriminatory power of PGC. A dual-column method has been reported in an effort to further improve the discrimination of PGC of paint samples (248). This method uses a polar and a nonpolar capillary column connected to the same injection port of a gas chromatograph. The pyrolyzate vapors are split between the two columns, and a separate, different pyrogram is generated simultaneously for the same sample.

Figure 16.22 shows a typical pyrogram from a paint sample using a single capillary column. The repeatability of this system is good while also providing good discrimination power. Table 16.16 lists the chromatographic and pyrolysis conditions that were used to generate this pyrogram.

In 1978, Audette and Percy introduced the idea of first examining a paint chip by infrared spectroscopy (IR) using a KBr pellet and then pyrolyzing the same pellet for gas chromatographic analysis (249). This method offers high discriminative power with the use of two distinctly different techniques and a very small amount of sample (3–5 μg).

More recently, PGCMS has been evaluated in the analysis of the new paint formulations (250). PGCMS has also been used in combination with Raman spectroscopy for the characterization of automobile paints (251,252a). Laser micropyrolysis GCMS has been used for the analysis of paint, photocopier toners, and synthetic fiber materials (252b). This emerging technology uses a laser microprobe to selectively target very small areas of materials for GCMS.

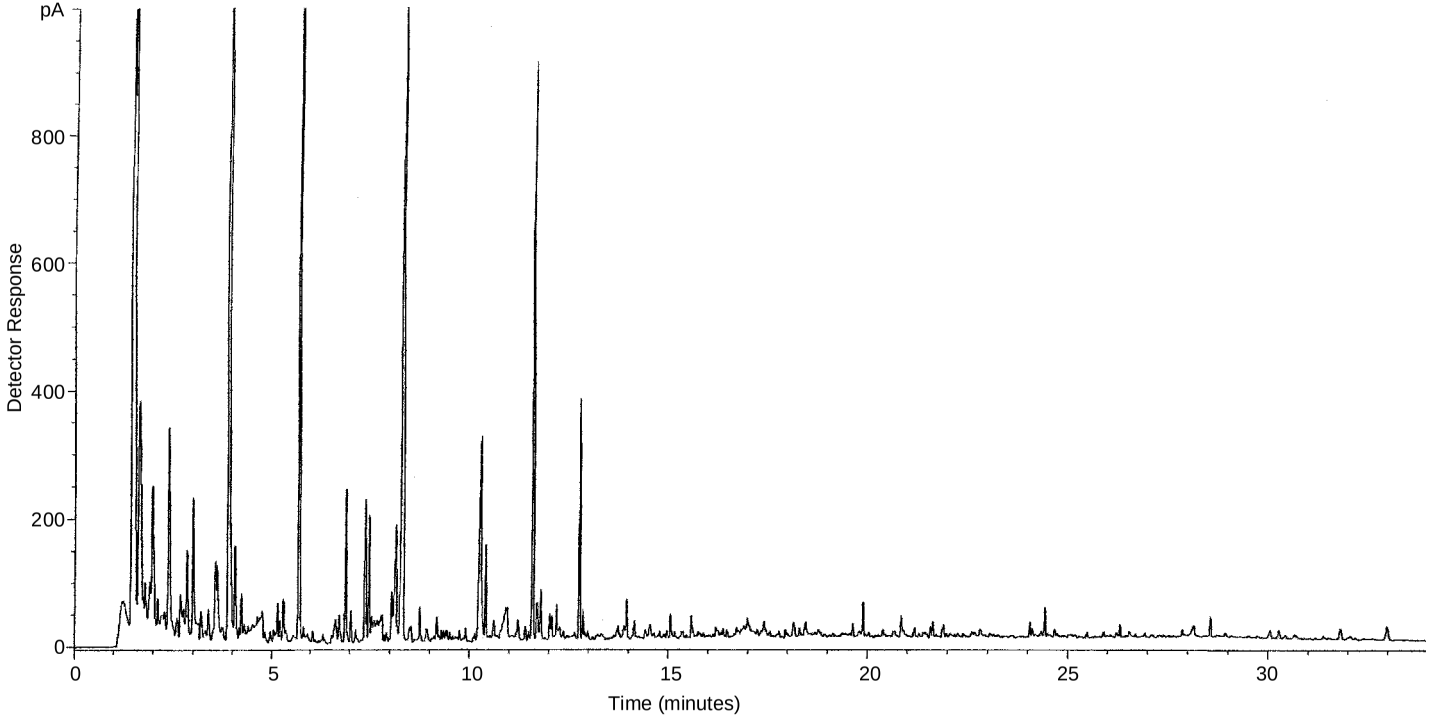


FIGURE 16.22 Pyrogram of a red acrylic enamel automobile paint resulting from the pyrolzolate being split onto a 30-m HP-5 capillary column. GCFID conditions: see Table 16.16. (Courtesy of New Jersey State Police.).

TABLE 16.16 Conditions for PGC of Paints and Fibers[a]

Sample	Red Acrylic Enamel Automobile Paint (1 mm^3)
Column	HP-5 30 m × 0.32 mm i.d. (0.25 μm)
Oven temperature	40°C(2 min) to 295°C at 10°C/min
Injection temperature	290°C
Detector temperature	295°C
Detector	FID
Pyrolysis temperature	700°C
Pyrolysis duration	20 s
Interface temperature	200°C
Linear gas velocity	30 cm/s, helium
Split ratio	30/1

[a] See Figures 16.22 and 16.23.

16.11.3.2 Fibers

The identification and comparison of fiber evidence in the forensic laboratory can encompass a number of different techniques. Nondestructive examination of fibers by polarized light microscopy, microspectrophotometry, and FTIR spectroscopy very often provide a sufficiently high discrimination in most casework comparisons that other analytical tests are not needed. When a sufficient amount of fiber evidence exists, a comparison of fiber dyes by thin-layer chromatography (TLC) or HPLC will often further the discrimination. Auxiliary techniques such as PGC, PGC/MS, pyrolysis mass spectrometry (PMS), and SEM-EDXS are only sporadically used when the evidence or situation is required.

When considering this scheme of fiber comparisons, it is not surprising, to find that the literature is not as abundant with PGC applications of fiber identification as is the case for paint evidence. This is disappointing, since there is ample evidence that PGC can distinguish between various types of fibers, such as polyesters, acrylics, nylons, cellulose, and acetates (253–256). The discrimination power of PGC for distinguishing various types belonging to a single fiber class, however, is a subject of question. Bortnial et al. in 1971 examined a number of acrylics and modacrylics and were able to differentiate various types of nylon fibers, distinguishing, for example, nylon 66, 11, 610, 6, and Nomex (257). Almer analyzed polyacrylonitrile fibers, 63 acrylic and 22 modacrylic, with a Pyroprobe and capillary column using as little as 10 μg of sample (258). In a review, Wheals concluded that PGC was slightly less effective than IR spectroscopy for discriminating fibers and that the conditions normally used for the analysis of paint chips was unsuitable for fiber comparisons (259).

Figure 16.23 shows a pyrogram resulting from the pyrolysis of a polyester fiber. The conditions used to analyze this particular fiber (listed in Table 16.16) are typical of conditions used to analyze a variety of different fibers and are similar to the conditions used for PGC of paints (Table 16.16).

There have been some other forensic applications of PGC of fibers. Levy and Wampler used a Pyroprobe and a capillary column to examine various synthetic

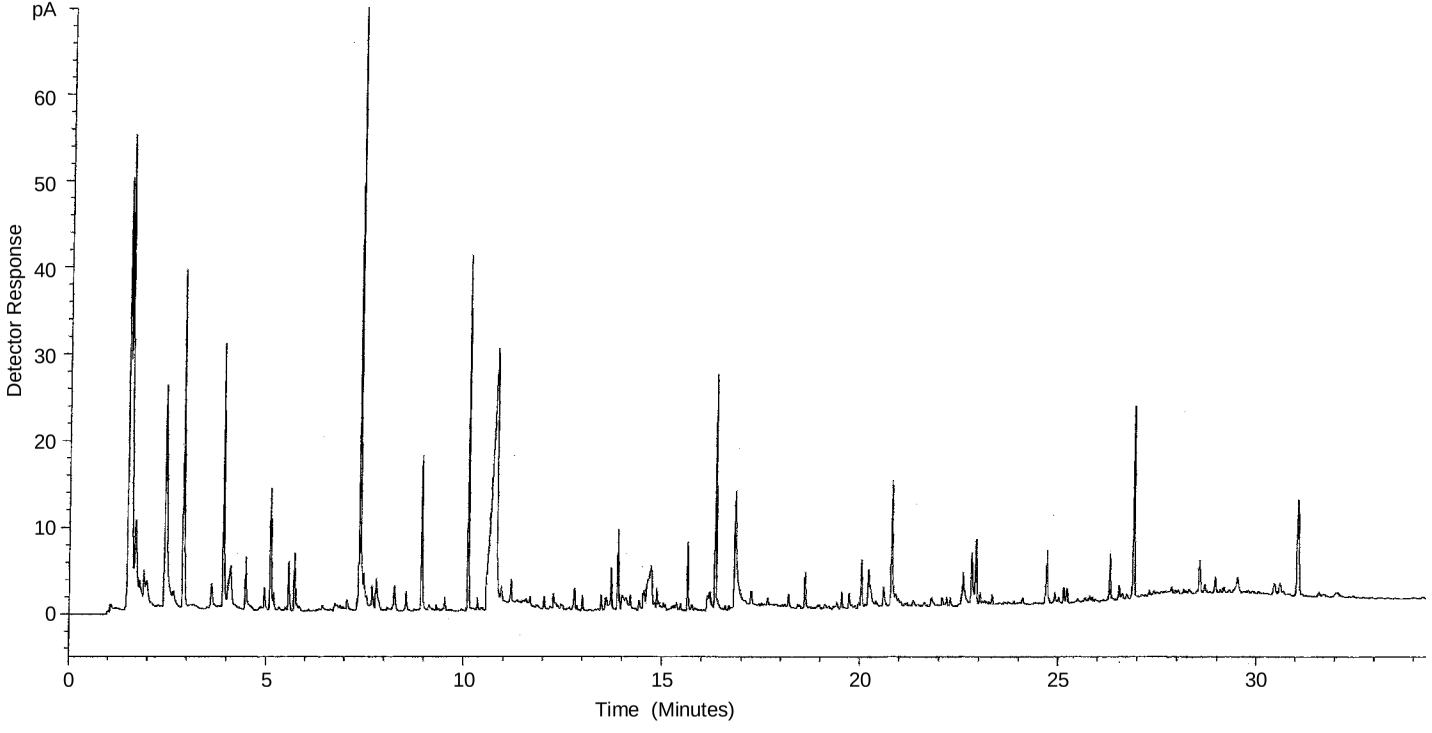


FIGURE 16.23 Pyrogram of a 1-cm length of a polyester fiber on a 30-m HP-5 fused-silica capillary column (0.32-mm-i.d. × 0.25-μm film): GCFID conditions: see Table 16.17. (Courtesy of New Jersey State Police.).

fibers (260). Hardin and Wang used a Pyroprobe with quartz sample tubes and packed-column GC to examine several types of textile fibers (261). Wright et al. used a microfurnace pyrolysis (MP) unit interfaced to a capillary column gas chromatograph to pyrolyze various synthetic fibers (262). They used multidimensional techniques, including heartcutting and cryofocusing, to obtain better resolution and discrimination of the fibers. A study of the effects of a soil environment on the biodeterioration of manmade textiles showed that fibers could still be identified by IR spectroscopy and PGC as to fiber type after 12 months of exposure, even though the solubility behavior and optical properties (polarized light microscopy and dispersion stains) both had changed (263).

More recently, PGC was used to identify minute samples of wool fiber (264).

16.11.3.3 Other Polymers

PGC has been applied to the characterization of a number of different types of polymers besides the traditional paint chips and fiber evidence submitted to the crime laboratory. Synthetic plastics have been readily identified and differentiated by PGC (253). Hume et al. (265) applied PGC to the characterization of motor vehicle body fillers utilizing a 15-m Carbowax 20 M gas chromatographic column and a computer-based system to compare pyrograms.

Davis (266) used a Pyroprobe with quartz tubes to examine rubber from a truck tire, the handle of a hammer, and an automobile bumper guard. Blackledge (267) used PGC to compare a rubber bumper guard removed from a suspect car to one recovered at a hit-and-run scene. Both investigators used a different approach than the traditional high-temperature PGC. The polymer samples in both cases were first heated by the injection port of the gas chromatograph to produce a chromatogram of the rubber's volatile components, and then the sample was pyrolyzed. This approach first reported by Chih-An Hu (268,269) is really a combination of thermal desorption and PGC and has the potential for further increasing the power of discrimination of both techniques.

Lennard and Margot (270) examined and compared traces of synthetic material from shoe soles by FTIR microspectrometry and packed-column PGC. Ding and Liu (271), in a similar analysis, examined 10 different rubber-soled shoes and 12 different automobile tires by Curie point PGC using two separate packed columns. They used varying ratios of butadiene–styrene, natural rubber, and butadiene–acrylonitrile to differentiate the samples.

16.11.3.4 Miscellaneous Applications of Pyrolysis Gas Chromatography

Beyond paints, fibers, and other polymers, PGC has been applied in the forensic science laboratory to characterize and compare a number of different types of material submitted as evidence in criminal casework. The utility of PGC for the characterization of adhesives has been described (253,272), as well as various methods for the comparison of tapes with adhesive backings (273). Vinyl tile with an asphalt-type glue from a safe-cracking case was analyzed by PGC (274). Williams and Munson (275) used capillary column PGC to examine 30 black

PVC tapes, distinguishing 26, and even partially burned tapes could be examined. The analysis of photocopy toners by PGC has been reviewed (276), and it has been demonstrated that PGC is a useful technique for the differentiation of photocopy toners (277,278).

Newlon and Booker (279) applied PGC to the identification of smokeless powders. They were able to differentiate 40 samples on the basis of their chemical composition. However, Keto (280) examined smokeless powders by capillary-column PGC and concluded that PGC has only limited value for the source identification of smokeless powders. A comparative study of samples of chewing gum bases has been conducted using PGC (281). Criminalists at the police crime laboratory in Osaka, Japan used capillary PGCMS to "fingerprint" Japanese cedar, Japanese cypress, and American pine (282). A pyrolysis derivatization technique involving copyrolysis with tetramethylammonium hydroxide was used to characterize heartwood lignocellulose from selected softwoods and hardwoods (283). Rosin-based resins were characterized by pyrolysis and simultaneous pyrolysis methylation GCMS techniques (284). The application of PGC to the detection of art forgeries has been described (285).

16.12 MISCELLANEOUS FORENSIC APPLICATIONS OF GAS CHROMATOGRAPHY

The aforementioned applications of GC have been from several major areas of forensic science, specifically drug analysis and toxicology and other areas of trace evidence, such as pyrolysis, explosives, and the detection of accelerants. There are, however, a few other forensic analyses by GC that have been reported.

In 1985, a gas chromatographic procedure for comparing the relative ages of ballpoint inks was reported (286). This procedure is based on the quantitative analysis of solvents contained in the inks, which are reported to remain in the paper for up to one year. The technique involves extracting the dried ink on paper with strong solvents, such as pyridine, and then performing a quantitative analysis by GC.

The quantitative determination of oxalic acid in biological fluids is critical for the effective diagnosis of fatal ethylene glycol or oxalic acid intoxications. In 1980, a procedure was described that is a specific and quantitative micromethod for the determination of oxalic acid in forensic specimens as its 2,3-trimethylsiloxyquinoxaline derivative using GCMS (287).

In 1981, GC was used to determine the racemization of aspartic acid in dentin for the estimation of age. The *N*-trifluoroacetyl isopropyl esters of amino acids in dentin from teeth were quantitatively compared to estimate age (288).

A thermal desorption capillary gas chromatographic method has been use to analyze volatiles from clingfilms (289). This procedure was able to differentiate 14 brands of polyvinylchloride film and 7 brands of polyethylene film. The forensic examination of clingfilms is often requested, and this gas chromatographic procedure offers an alternative to physical methods for comparing control and recovered clingfilms.

The detectability and stability of lachrymators were investigated using dichloromethane extraction followed by GCMS (290). The chemical nature of fingerprints, to ascertain whether differences in chemical composition or the existence of chemical markers can be used to determine personal traits, such as age, gender, and personal habits, was investigated using fingertip residue extracted by chloroform and analyzed by GCMS (291). High-temperature GC has been used to analyze the wax or artificial fireplace logs (292).

These few examples should give the readers some idea of how GC is used in forensic science. For more examples or a thorough review of the topic the reader is referred to the literature on forensic science (293).

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CHAPTER SEVENTEEN

Validation and QA/QC of Gas Chromatographic Methods

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17.1 INTRODUCTION

As with any equipment, the gas chromatograph requires validation before being used in an analytical method. Validation is a critical part of the development of an analytical gas chromatographic procedure. It is what demonstrates accuracy and precision. Validation is defined as "establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes" (1). It is generally concerned with a specific analysis or method. Validation of a method, however, begins way before samples can be prepared. It begins with the installation and qualification of the instrument and continues with good quality assurance/quality control (QA/QC) in the laboratory.

Quality assurance is a system of activities whose purpose is to prevent problems from occurring and provides assurance that the instrument meets defined standards of quality with a stated level of confidence. The International Organization for Standardization (ISO) provides a general guide for quality assurance programs and analytical laboratories are using these more. ISO Guide 17025 is the standard that many analytical laboratories, including crime laboratories, follow.

While most laboratories realize the need for a good QA/QC program, it is seldom clear which program meets the needs of a particular laboratory, especially if that laboratory is performing a wide variety of analytical services. QA/QC, therefore, can have different meaning to different chromatographers depending on their particular area of interest and standards. However, there are basic similar key elements to a good quality assurance program in gas chromatography that must be addressed. These are designed to ensure that the sample was free from all possible contamination, that it was handled appropriately by trained analysts using calibrated and maintained gas chromatographs and standard validated procedures, and that all of this was appropriately documented. The key elements to a good QA/QC program for gas chromatography will be discussed in greater detail throughout this chapter.

17.2 PROCUREMENT

The first step to ensure quality of the gas chromatograph is the procurement itself. Capabilities and specifications of gas chromatographs vary. Manufacturers

publish technical specifications for their instruments, but those specifications may not be meaningful to your application. They may also include unique, and sometimes useless, characteristics that can be used to preempt another manufacturer from meeting that specification. It is important to procure the correct instrument for your needs. Do you need an instrument with high sensitivity, high precision, or versatility? Is it important to be able to accommodate dirty samples, a variety of matrices, and long runtime between calibrations? What types of injectors and detectors are best for your work? The best way to get the correct instrument is through a performance specification. Determine the realistic performance characteristics that are important to your analyses, and include them as requirements in your procurement document. Examples of performance specifications include the following:

Sensitivity—1 pg of heptachlor epoxide must give a response (peak height) greater than 50 times the background noise level.

Carryover—the peak area from an injected blank must be less than 0.001% of the peak area of a 100-μg/mL ethanol injection that immediately precedes the blank injection.

Linearity—the response, measured as peak area, must be linear (correlation coefficient greater than 0.99995) over three orders of magnitude starting with the minimum detection level (3 times the baseline noise).

Autosampler precision—relative standard deviation of peak areas of 20 consecutive injections must be less that 0.5%.

Calibration drift—the change peak area of a midrange calibration standard over a 6-h period must not exceed 2.0%.

Each instrument performance characteristic that is important to the application should be identified in the procurement specification. The specification will become part of the bid package that is sent to the vendors. In the bid package also state that the vendor must provide evidence with the quotation that the proposed instrument can meet those specifications, and on installation of the instrument, the manufacturer's installer must demonstrate that the instrument installed at your location meets those specifications.

17.2.1 Facilities

The facilities that house gas chromatographic instrumentation are extremely important for producing quality, reproducible data. The design of the laboratory, security, and storage are issues that must be addressed. The laboratory must have a documented security policy that ensures the integrity of the samples by limiting access to areas affecting the quality of testing. Separation of activities that might cause cross-contamination is a prime consideration. Also, control of the environment, especially such variables as temperature and humidity, is extremely important for reproducibility. These are factors that could influence the validity of the test results. Make sure that the temperature and humidity of the laboratory

fall within the instrument's specified guidelines. Knowing the BTU output of the instrument will help estimate the load for the laboratory's heating, ventilation, and air-conditioning (HVAC) system.

Samples should be stored under suitable conditions to inhibit sample degradation and loss of analyte. If refrigeration or freezing is necessary, then the documentation of proper operation and continuity of operation of the refrigerators/freezers is important for a good quality program.

17.2.2 Installation and Setup

Instrument installation and setup is critical to obtaining the best results in gas chromatography. Without the right equipment, supplies, facility, and support system, the operator is running the risk of compromising the instrument's potential to deliver the desired performance. Before installation of a new gas chromatograph the operator should obtain preinstallation checklists from the instrument manufacturer and follow the directions. The checklists and technical specifications are a valuable source of information, not only about the instrument but also for supplies and accessories. The correct gas supply is of utmost importance. The right-grade carrier and detector gases must be used from a reliable source that is compatible and recommended by the manufacturer. Using lower-quality gas with additional filters and purifiers is not recommended. Dual-stage regulators rated for gas chromatography and are labeled "high purity" should be used as well as proper gas filters (2). The reader is referred to instrument manufacturer publications for more information on this subject (3).

17.2.3 Installation Qualification (IQ)

The qualification process has many different facets such as the facility and logistics of the instrument, the equipment support systems, the gas chromatograph, and the analytical method. Installation qualification (IQ) generally involves the instrument installation by the vendor and in some cases is usually required by the vendor to validate the warranty of the instrument.

Acceptance Testing Although having the manufacturer install the instrument costs more, it is money well spent. It puts the burden of initial quality and correct initial operation on the manufacturer.

The performance specifications that were included in the bid package are now the criteria for acceptance of the instrument. Any defects in the instrument, installation errors, or overall quality issues with the instrument will be evident if compliance with the specifications cannot be demonstrated on site. It is important that documentation of the initial performance be retained for future reference.

Instrument qualification is the process by which the gas chromatograph is checked for compliance with previously determined standards of function and performance. This is best accomplished by having the vendor (or the person

doing the installation) demonstrate this performance onsite with a test mixture on the column provided by the manufacturer. This performance test chromatogram should then be made a permanent record of the instrument log.

Some regulatory agencies require that the vendor have a documented and certified quality assurance system in place such as ISO 9001. The vendor under this program must also validate instrument software. This ensures that the quality of the product(s) will meet the vendor's published specifications and claims for performance.

17.2.4 Operational Qualification (OQ)

Operational qualification (OQ) occurs when the gas chromatograph is tested and shown to meet specified requirements of the method and/or reproducing a test chromatogram provided by the manufacturer of the chromatographic column. Laboratory personnel usually demonstrate instrument operational qualification by reproducing the vendor's OQ performance chromatogram of the test mixture on the column provided by the manufacturer. This performance test chromatogram should then be made a permanent record of the instrument log. Often this test mixture is the Grob mixture (see Figure 3.24), which tests for active sites on the column. The Grob mixture does not ensure resolution or specificity of the particular method. A particular resolution mixture for the method should be evaluated for this purpose.

17.3 PERFORMANCE QUALIFICATION (PQ)

Regulations such as Good Laboratory Practice (GLP) and Current Good Manufacturing Practice (cGMP), quality standards and guidelines, such as EN45001 and ISO Guide 25, require the performance of gas chromatographs to be routinely controlled. This step is generally referred to as performance qualification (PQ) or performance monitoring. This is an on-going process performed by laboratory personnel. Instruments should be tested for suitability before and during routine use and quality control checks should be built into routine analysis to verify analytical results for accuracy. This takes time for both the operator and the instrument to perform these tests. If the quality assurance checks are done at the beginning of every day, this does not guarantee the performance over the full day, so QA samples need to be designed intermittently into the procedures. PQ must be conducted throughout the life of the instrument in order to demonstrate that the gas chromatograph consistently performs up to the specifications for each analyte that will be analyzed using the instrument.

Critical parameters such as the resolution between two peaks of choice, the baseline noise, or a compound's response should be determined during the method development. Software programs that automatically measure and monitor these values can be developed by an individual laboratory (4) and are also available commercially.

17.3.1 Service and Maintenance of a Gas Chromatograph

Scheduled service repairs and routine periodic maintenance of gas chromatographs are a critical part of a good quality assurance program and will save time in the long run. A well-maintained instrument will give more reliable and reproducible data, and the lifetime of the instrument will be extended.

Preventive maintenance anticipates problems that are not or cannot be handled during routine maintenance and may take several days to accomplish, for example, detector or injector maintenance. The operator can plan for preventive maintenance during a lull in the workload or a prescheduled time. Three simple tasks are often overlooked and can prevent problems in gas chromatographs: (1) inspect and change autosampler syringes, (2) change septa regularly, and (3) inspect and change inlet liners frequently. Autosampler syringes are prone to far more complications than manual syringes. The operator can avoid or at least delay these problems by taking several precautions (5). Inlet septa are weak spots in the gas chromatographic pneumatics. Because syringe needles puncture septa numerous times, septa particles build up in the injection system and can cause numerous chromatographic problems. A routine schedule of septum changing and inlet liner inspection is a necessary part of operation. A more comprehensive treatment of these and more related issues to gas chromatograph maintenance can be found in other sources (6,7).

17.3.1.1 Original Quality Replacement Parts

When servicing the instrument it is very important to replace the original parts and accessories with quality replacement parts. The operator should make every attempt to purchase original manufacturer replacement parts or parts that have the same or better quality and meet the technical specifications provided by the manufacturer of the instrument. Replacing inferior parts and accessories in the gas chromatograph will only lead to problems later on and may cause more serious damage to the instrument. This will only lead to more downtime and less productivity. Manufacturers product literature is a very good source of information for this issue. There are also companies that purchase overstocked parts and accessories as well as items that may not be supported directly for service by the manufacture. It is then possible to purchase original quality replacement parts from these sources.

17.3.1.2 Verification after Service

Verification that the gas chromatograph is operating properly after service must be documented. The verification can be accomplished by testing the gas chromatograph and showing it to meet previously tested (usually be vendor or person installing instrument) specifications. The laboratory personnel usually demonstrate instrument qualification by reproducing the vendor's IQ performance chromatogram of the test mixture. This performance test chromatogram should then be made a permanent record of the instrument log.

17.4 PERSONNEL AND TRAINING

All employees should have a personnel file that documents the qualifications (resume) and training for the job. All personnel should have a training folder in this file. For employee development, their supervisor should review both the training folder and resume annually. Employees should be comfortable in their knowledge of gas chromatography and how to use a gas chromatograph. Once trained, the employee must show proficiency by demonstrating operation under supervision. Once the supervisor is satisfied that the procedure has been learned, a document should be signed and dated certifying training and proficiency.

17.5 STANDARD OPERATING PROCEDURES (SOPs)

17.5.1 Preparation, Modification, and Revision of SOPs

All procedures in the laboratory including sample preparation, analysis, and reporting must have a written standard operating procedure. Any deviations from this procedure for whatever reason must be documented and approved. Should it be necessary to revise the SOP the reason should also be documented and the original procedure archived. Employees should know via written documentation how much they are empowered to vary all procedures used.

In the United States, the Good Laboratory Practice (GLP) standards regulate most programs including pharmaceuticals, environmental analysis, and the cosmetic industry. Additionally, regulatory agencies such as the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) demand additional and specific requirements for analytical procedures that employ among other instruments, the gas chromatograph.

17.5.2 Validation of Gas Chromatographic Methods

Method validation is the process of proving that an analytical method is acceptable for its intended purpose (8). Since gas chromatographic methods are used for different purposes, the method validations may also be different. For example, several publications outline guides to validate pharmaceutical methods such as the *United States Pharmacopoeia* (USP) (9), International Conference on Harmonisation (ICP) (10,11), and the Food and Drug Administration (FDA) (12,13). In general, methods must include studies on selectivity, linearity, accuracy, precision, dynamic range of response, limit of detection (LOD), limit of quantification (LOQ), and robustness. Although there is general agreement about what type of validation studies that should be done, there is great diversity in how they are to be accomplished. The literature contains a variety of approaches to performing validation studies using gas chromatography (14–17). This chapter presents an approach that serves as a basis to perform validation studies for most gas chromatographic methods.

17.5.2.1 Minimum Criteria

The first step in the development and validation of a gas chromatographic method should be to set clear and understandable minimum requirements that are acceptable to the chromatographer and to the end user. A complete list of criteria should be made and evaluated before the method is validated. For example, the method precision should be ±2% and the method should be accurate to within 3–5% of the target concentration. Examples of suggested criteria may vary but a specific application may call for additional studies. The statistics generated from the validation studies should be similar and predictive of the range of values gathered from real sample analysis. During the actual studies and in the final validation, the minimum criteria will set clear goals for acceptability standards of the method.

17.5.2.2 Selectivity

Selectivity is a measure of the capability of the analytical method to determine a particular analyte in various matrices with minimal or no interference from other matrix components. *Specificity* is a measure of the capability of the analytical method to be perfectly selective for an analyte or group of similar analytes. Specific gas chromatographic methods are not common; the more common description of a gas chromatographic method is that it is selective. Usually specificity comes from combining chromatographic methods with more specific detectors such as GC/mass spectrometry (GCMS) or GC/infrared spectroscopy (GCIR).

The validation of a chromatographic method must involve demonstrating selectivity of the method with the ability to measure the analyte response in the presence of all potential sample components. Before any sample is introduced into a chromatographic system, the appropriate resolution criteria must be outlined and satisfied. For example, selectivity criteria for a method will outline the baseline chromatographic resolution from all other sample components. This should be verified by analyzing a resolution mixture with appropriate components. Figure 16.15 shows the resolution mixture for the analysis of ethanol in blood. Ethanol is baseline resolved from all of the other components.

The ability to resolve individual compounds is generally the limiting factor for the number of analytes that can be measured using a single procedure. It is good practice to list analytes that may not be resolved from one another. If appropriate resolution cannot be achieved, the unresolved components at their maximum expected concentrations should be validated to demonstrate that these components would not affect the final results. An alternative method would be to report the sum of the two (or more) unresolved analytes (e.g., *m*-xlyene + *p*-xlyene). If an alternate column is to be used to increase the selectivity, then this should be identified and similar validation data should be collected for this column.

17.5.2.3 Initial Calibration

Calibration of the gas chromatographic system is another critical step in the generation of quality data. Calibration of the gas chromatograph delineates the relationship between the detector response and the concentration of the analyte

introduced into the instrument. A calibration curve is the graphical depiction of this relationship. In order to perform quantitative measurements, a calibration curve must be established prior to any analysis of samples, and therefore, is termed initial calibration.

Most methods rely on a linear calibration curve, where the detector response is directly proportional to the amount of the analyte. Unfortunately, at times, the method cannot be optimized for all of the analytes to which it is applied, thus the analyst is forced to us a nonlinear calibration curve. Generally, the nonlinear calibration option is necessary to achieve low detection limits or specific analytes in a unique methodology. This should be avoided if at all possible.

Initial calibration of a gas chromatographic method involves the analysis of standards containing target compounds at different concentrations covering the working range of the instrument. In order to produce acceptable sample results, the detector response must be within the working range established by the initial calibration. The extrapolation of the calibration to concentrations above or below those of the actual calibration standards is not appropriate and may lead to error. The chromatographic system may be calibrated using either the external or the internal standard techniques described in Chapter 8. Regardless of which method is used, the calibration standards should be introduced into the gas chromatograph using the same technique that is used to introduce the actual samples.

17.5.2.4 Calibration Linearity

Validation of a chromatographic method demands that linearity be established to verify that the analyte response is linearly proportional to the concentration range of interest. Chromatographic methods allow the use of both linear and nonlinear models for the calibration data. Given the limitations of the nonlinear method, the analyst will most likely choose the linear model. The nonlinear calibration model may be necessary to achieve very low detection limits or to address specific method demands.

A linearity study is generally performed by preparing analyte solutions at various concentration levels. Standards should be prepared and analyzed a minimum of 3 times. The standards are then evaluated using the chromatographic conditions for the method. In the final method procedure, three standards are generally used but in some instances, a single standard concentration is used to verify linearity. Validating over a wide range provides confidence that the routine standard levels are well removed from nonlinear response analyte concentrations.

The simplest approach to establishing linearity is to calculate both calibration factors and response factors as a measure of the slope of the calibration line and assume the curve passes through the point of origin. Under ideal conditions, the factors will not vary with the concentration of the standard that is injected into the gas chromatograph. In practice, some variation is to be expected. Acceptability of linearity data is often judged by examining the correlation coefficient and the y intercept of the linear regression line for the response versus concentration. A correlation coefficient of >0.999 is generally considered acceptable. The y intercept should be less that a few percent of the response obtained from the target level (8).

17.5.2.5 Accuracy

Accuracy is how close the experimental data is to the "true" value. Accuracy is a very difficult parameter to measure or validate because the analyst must consider sampling errors, errors in procedure workup, and errors from separation interferences, and the detector system. Another important problem is obtaining standards for the analyte in the matrix to be analyzed. National Institute of Standards and Technology (NIST) reference standards are often used, but not always available for all analytes of interest. Environmental, biological, and forensic matrices are especially difficult to reproduce. Standard Addition Techniques and matrix spikes may be used and recoveries determined. The Standard Addition Technique gives one an indication of how the method performs with respect to accuracy of the final procedural steps. This approach is used if it is not possible to prepare a blank sample matrix without the presence of the analyte. A good test for accuracy is to compare the new method with a totally independent method of known accuracy. The accuracy should be evaluated at the low as well as the high analyte concentration values expected. An example of an accuracy criterion for a particular method is that the mean recovery value will be 95 $\pm$2% at each concentration over 80−120% of the analyte range concentration.

17.5.2.6 Precision

The precision of a gas chromatographic method is the measure of agreement or closeness of analyte concentrations to each other when the analyses were performed using identical conditions, i.e. the same method, same sample, same operator, and same laboratory conditions over a short period of time. This is known as *repeatability*. This is generally the measure of the amount of scatter in the results obtained from multiple analyses of a sample. Mathematically it is calculated and expressed as standard deviation (SD).

$$\overline{x} = \frac{x_1 + x_2 + x_3 + \cdots + x_n}{n}$$

$$\mathrm{SD} = \left[\sum \frac{(\overline{x} - x_1)^2 + \cdots + (\overline{x} - x_n)^2}{n - 1} \right]^{1/2}$$

The relative standard deviation (RSD) is defined as

$$\mathrm{RSD} = \frac{\mathrm{SD}}{\overline{x}} \times 100$$

The precision data is generally obtained from triplicate analyses of spiked samples and can be calculated from the accuracy study. The precision of the gas chromatograph and the precision of the method are two different values, and a good chromatographer should calculate both. The precision of the method can be obtained as above by calculating the repeatability of the method. The precision of the instrument can be obtained by multiple injections, usually 10, of one sample solution.

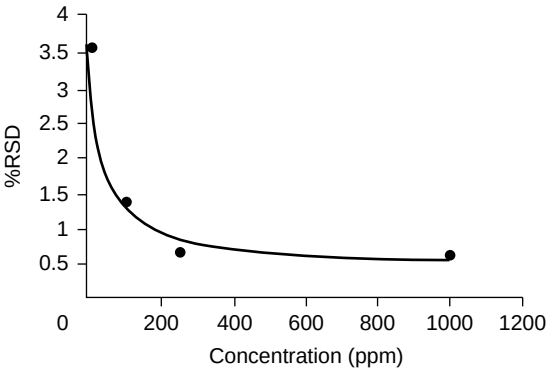

FIGURE 17.1 %RSD versus concentration for GC headspace analysis of ethanol (reprinted with permission from Reference 8).

Figure 17.1 illustrates how precision may vary as a function of analyte concentration. The %RSD values of ethanol quantification by GC increased significantly as the concentration decreased from 1000 ppm to 10 ppm. Higher variability is expected as the analyte levels approach the detection limit for the method (8). The analyst must determine during the validation of the method at what concentration the imprecision becomes too great for the intended use of the method.

An example of precision criteria for a gas chromatographic method would be that the instrument precision (RSD) should be ±1%, and the repeatability of the method would be ±2%.

A distinction between repeatability and reproducibility should be made here. *Reproducibility* is data collection using the same sample and the same method but a different operator, another set of laboratory conditions, and a different period of time (days or even weeks). (*Note*: It is possible for one analyst to *reproduce* or even *repeat* their own data, but a second analyst can only *reproduce* the first analyst's data.)

An example of the reproducibility criterion for a gas chromatographic method would be that the results from multiple laboratories should be #2% of the value obtained by the primary testing lab.

17.5.2.7 Range of Method

The better chromatographic method provides linearity, accuracy, and precision over a substantial range of concentration. In validation, the range of the method is generally demonstrated beyond the boundaries of the working range of analyte concentrations. The working range of the method generally gives an optimum concentration range for quantitative analyses.

In practice, the linear range is generally determined by analysis or samples of varying concentrations of the analyte of interest and plotting concentration versus detector response. This is done as previously described for determination of accuracy, precision, and linearity.

17.5.2.8 Limit of Detection

The *limit of detection* (LOD) or detection limit of a method is the lowest analyte concentration that the detector will produce a response detectable above the background, or noise level, of the system. The *minimum detectable level* (MDL) is the concentration level at the LOD and generally defined as three times the noise level (baseline) of the detector. LOD and MDL are the two quantifiable values that can measure the sensitivity of the method. *Sensitivity* is the smallest difference in the response of the detector (signal) that can be detected for the method. LOD is the smallest amount that is clearly distinguishable from the background or blank.

Two methods are commonly used to determine LOD: a statistical approach (18,19) and the empirical or experimental approach. The statistical method is an easy way to generate an LOD, but that value is likely to be artificially low. The experimental LOD method produces a value that represents the real limit of feasibility of a method and value that meets all routine analytical acceptance criterion. It is more logical to progressively decrease analyte concentration until the acceptance criterion fail than to measure blank samples and extrapolate upwards data that are doomed to fail the requirements of the method (20). The detection limits are determined by repeated analyses of a blank matrix (no analyte of interest present) and a matrix containing the analyte of interest at a known concentration (21). The chromatographer should not determine detection limits in reagent blanks. This will eliminate matrix effects and give a false MDL. It is also important to test the method on different gas chromatographs such as those used in different laboratories to which the method will be transferred.

17.5.2.9 Limit of Quantification

The *limit of quantification* (LOQ) is the lowest level of analyte that can be accurately and precisely measured with the method. This parameter should be measured in the matrix. The LOQ must always be measured by experimental data and not by extrapolation of experimental data. Generally, an acceptable LOQ is 10−20% of the RSD. The RSD is also called the *coefficient of variation* (CV).

17.5.2.10 Ruggedness of Method

The chromatographer must be certain that the new method holds up under other conditions than what the method had been validated. This may be determined by establishing that the analyte(s) of concern are stable during storage conditions, throughout the method preparation phase, and in the standard prepared sample. This is considered the *ruggedness* of the method.

17.5.2.11 Robustness of Method

The *robustness* of a method is the ability of the method to allow the analyte to remain unaffected by small changes in parameters such as ionic strength of the sample, detector temperature, temperature of the sample, and injection volume. These method parameters may be evaluated one by one or altogether during on experiment. Obtaining this data allows the chromatographer to become knowledgeable about the limitations of the method.

An example of one method to control the robustness and reproducibility of a method is to set retention time windows for the target compounds. Absolute retention times are used for compound identification. Retention time windows can be established to compensate for minor shifts in absolute retention times as a result of sample loadings and normal chromatographic variability. The width of the retention time window should be carefully established to minimize the occurrence of both false positive and false-negative results. This can be accomplished by multiple injections of standard solutions over the course of a time period (days or weeks) and then calculating the mean and standard deviation of the retention time.

An example of setting a retention time window criterion for a gas chromatographic method would be that the retention time of the target compound should be ±2.5% or the standard run on that particular day.

17.5.2.12 Software Validation

Most chromatography software is thoroughly validated by the manufacturer prior to distribution. When it comes to quantification, however, the validity of the integration is important to accurate results. On the chromatogram, the quantity of analyte is proportional to the area under the peak for that analyte. The accurate calculation of that area is critical to achieving a valid calibration.

Not all peaks are ideal in shape; that is, they do not always have a Gaussian distribution. The integration software provided with the chromatography data station calculates the area under the peak of interest. When a Gaussian peak is encountered, the integration is easy, but often there are complications due to overlapping peaks, analytes that elute on the tail of the solvent peak, baseline noise, asymmetric peaks, and other anomalies. Obviously the best way to deal with these issues is to improve the chromatography, but that is not always fully effective. The peak sensing and integration software must then be used to optimally determine the peak area. Often the default parameters for peak sensing and integration work well, but when the chromatogram is complex, operator intervention is usually necessary to optimize the integration.

Most integration software provides for identifying the baseline treatment used to determine the peak area. These treatments usually indicate the points used for the baseline at the start of the peak and at the end of the peak (Figure 17.2). For example *baseline to baseline* indicates that the instrument response was at the baseline when the integrator sensed the peak and the response returned to baseline after the peak. *Baseline to valley* indicates that another peak was sensed before the first peak returned to baseline. The minimum response between the peaks was used as the separation point between the peaks and the baseline that was used was that minimum response point. But perhaps it would be more appropriate to use the horizontal extension of the baseline instead of the valley point when calculating the area of those peaks. That is where optimization comes in to play.

Some baseline treatment indicators commonly used are

- *Baseline*—the peak started or ended on the instrument-calculated baseline.
- *Valley*—the inflection point between two peaks.

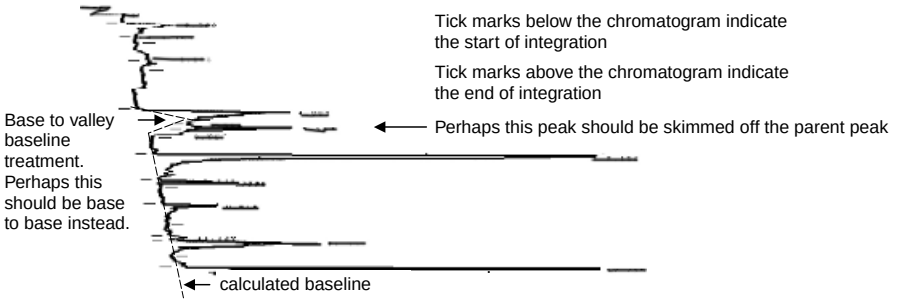
Tick marks below the chromatogram indicate the start of integration
Tick marks above the chromatogram indicate the end of integration
Perhaps this peak should be skimmed off the parent peak
Base to valley baseline treatment. Perhaps this should be base to base instead.
calculated baseline

FIGURE 17.2 Chromatogram showing integration points used to mark the baseline at the start of the peak and at the end of the peak.

- *Horizontal extension of baseline*—the baseline response has not returned but the area is calculated from the horizontal extension of the most recently determined base point.
- *Skim*—the peak is attached to the other much larger peak. The smaller peak starts and ends on one side of the larger peak before the larger peak returns to the baseline response. The smaller peak can be "shaved off" of the larger peak using the larger peak's interpolated response as the smaller peak's baseline.

Several variables can be set by the user that are used by the integrator to recognize the baseline and to recognize a valid peak's start and stop points. The variable's names vary with manufactures but generally accomplish the same tasks:

1. There is a variable that accounts for the baseline stability. Increasing this variable allows for a number of sudden but small changes in the response to have less effect on the baseline determination.
2. There is a variable that is used to limit the amount of long-term variation (drift) in determining the baseline. The larger this variable is, the easier it is for the integrator to determine a new baseline point that is at a different level from the previously tracked baseline.
3. There is a variable that is used to determine the magnitude of increasing response to identify the start of a peak. This is often used to eliminate some very small, broad peaks. The larger the value of this variable, the larger the response needed to trigger the system to recognize the increased response as the start of a peak.
4. The final variable is related to the rate of change of the response to trigger peak recognition. This is used to eliminate a gradually increasing baseline as being triggered as the start of a peak. The larger this variable, the less prone the system is to trigger on a slow increase of response. There are usually additional variables that can be user-adjusted to optimize peak sensing and integration. These may include the skim ratio and skim threshold variables to properly deal with coeluting and overlapping peaks.

To use these optimization variables effectively, the user must study the documentation provided with the software and become thoroughly familiar with the optimization variables provided. Generally there is a tool that allows the user to view the chromatogram with tick marks showing where integration starts and stops. This tool usually also provides the baseline treatment for each peak. By using these tools and adjusting the variables appropriately, satisfactory integration can usually be achieved. If there are some peaks that the user feels are still not integrated properly, usually there is a software mechanism to manually specify the start and stop points as well as the baseline to be used for an individual peak. After experimenting with the use of these variables for some time, the user generally can arrive at a set of variables that work for most chromatograms.

17.5.3 Sample Tracking and Chain of Custody

There must be a system in place that enables samples to be tracked through the laboratory. A chain of custody indicates who received the sample at the laboratory and when they received it, in addition to what and where the sample traveled within the laboratory. The documentation must show every step of the sample exchange, including who had control of the sample and where the sample was stored in the laboratory. If a computer tracking system is used, such as laboratory information management system (LIMS), then this also must be validated to demonstrate the system is reliable. Readers are referred to a guide for validation of a commercial LIMS, ASTM E2066-00, *Standard Guide for Validation of Laboratory Information Management Systems* (22). This guide is intended to educate individuals on LIMS validation, to provide standard terminology useful in discussions with independent validation consultants, and to provide guidance for development of validation plans, test plans, required standard operating procedures (SOPs), and the final validation report.

17.5.4 Statistical Process Control

Statistical process control has historically been used in manufacturing processes to anticipate problems. If the process performance is monitored at appropriate intervals, there will be an indication of performance degradation before the degradation is sufficient to have an adverse effect on the quality of the product. The process can then be halted, the cause for the condition corrected, and the process can be restarted. This can be applicable to gas chromatography as well. Any change in the output can be attributed to one or more changes in the process. The change may be deliberate or unintentional. If changes are recorded in a log, the relationship between the change and the performance may be correlated.

The key factors in successful statistical process control are selection of an appropriate control standard, selecting an appropriate monitoring interval, recording and analyzing the control standard results, and recording appropriate information in the instrument log. A control standard should be selected on the basis of its ability to accurately monitor the performance of the instrument. It should be sensitive to instrument performance, be available in large quantities, and be

stable over extended periods. Ideally you would want a standard that could be used for months or years on end. Because the addition of an internal standard to samples serves to correct for instrument performance variation, the use of an internal standard in the control standard can effectively mask many instrumental performance problems and defeat the effectiveness of statistical process control. The control standard should therefore not contain any internal standard. Each time the control standard is analyzed, its result should be added to the control chart, and the result analyzed within the context of previous analyses of that control standard. All pertinent data should be recorded in the instrument logbook. This would include gas cylinder changes, septum changes, all maintenance, column changes, and flowrate adjustments.

Normal variation is expected, and to discriminate between random variation and loss of process control, statistical analysis is performed on the results of the control standard. Random variation is expected and is out of the operator's control. Random variation is exhibited as distribution around the average (mean) of the values that conform to the normal distribution curve. The standard deviation of the results is an indicator of the level of control of the process. The smaller the standard deviation, the more controlled a process is. Statistically, in a controlled process approximately 95% of the values will fall within a range of the mean plus or minus two standard deviations, and 99.7% will fall within the mean plus or minus three standard deviations. Additionally when considered chronologically, the values should be randomly distributed on either side of the mean, and the further from the mean, the less frequent the occurrence.

A control chart is the best way to evaluate the results. After about 20 control standard results from a process that is in control are obtained, a statistical analysis can be performed. Calculate the mean and standard deviation. Plot a graph with the values on the vertical axis and the horizontal axis used for the chronology of the results (result 1, result 2; or use dates, times, etc.; see Figure 17.3). Plot

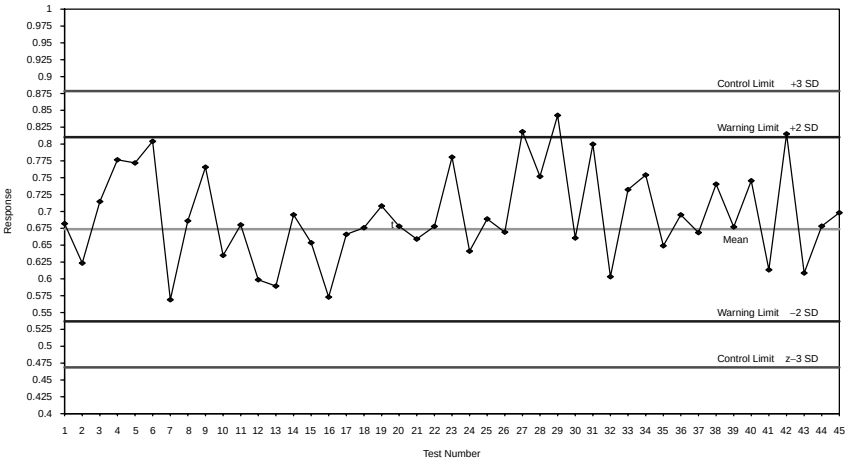

FIGURE 17.3 Graph showing a statistical process control chart.

the mean as a horizontal line corresponding to its value. Calculate the mean plus 2 times the standard deviation, and the mean minus 2 times the standard deviation. Plot those as horizontal line at the corresponding values. These two lines are called the "warning limits". Likewise, calculate and plot the mean plus and minus three standard deviations lines. These two lines are called the "control limits". Plot each control standard value on the graph. This is the statistical process control chart. As new results for the control standard are available, each one should be plotted immediately to determine if the process is in control.

Statistically, only 5 values in 100 should be between the warning limits and the control limits, and less than one in 300 should be outside of the control limits. If these levels are exceeded, it may indicate a potential problem. There are many other conditions on the control chart that indicate a potential quality problem. A few are listed below:

- Two of three consecutive values between the warning limits and control limits
- Values outside of the control limits
- Seven results on one side of the mean (central value)
- Distinctive trends up or down
- Several consecutive points that seem to establish a new central value
- 10 of 11, 12 of 14, 14 of 17, or 16 of 20 consecutive points on one side of the central line

Any of these conditions indicate nonrandom variation, and should be attributable to a process change. The process change may be determined by reviewing the gas chromatographic instrument logbook, or it may indicate that maintenance is necessary to restore the system to its proper operating condition.

As the process is improved through efforts to eliminate nonrandom variation, it will be necessary to recalculate the mean, warning limits, and control limits. If any control standard value indicates a potential quality problem, the analytical batch containing that control standard and all results since the last acceptable control standard should be considered of questionable quality and sequestered until an adequate quality evaluation can be completed.

17.5.4.1 Duplicate Analysis

Understanding that variation does occur in analytical results even when the analytical process is functioning properly, one must realize that at any time a quantitative result could be a random outlier (greater than three standard deviations from the actual value). Using only single analyses, outliers would be extremely difficult to detect unless the approximate value is already known. But by performing analyses in duplicate, an outlier can be detected because comparison of the results for the duplicate analyses would indicate a discrepancy. The probability of a random error in duplicate analyses being of the same magnitude and direction would be extremely low. The duplication of the analysis

should ideally be started at the beginning of the analytical process. If each of the duplicate samples is carried independently through all the processing steps (extraction, cleanup, derivatization, injection, etc.), the precision of the entire process can be evaluated and monitored. By calculating the relative difference (the absolute value of the difference between duplicates divided by the average of the two results), that value also can be monitored by a control chart. If the relative difference between duplicates exceeds the control limit, one of the duplicate results would be considered an outlier and the analysis should be repeated. Duplicate analysis is an extremely powerful quality control tool not only for gas chromatographic analyses but for most analytical techniques in general.

17.6 DOCUMENTATION

17.6.1 Written Instructions

The laboratory must have written instructions on the use of all pieces of equipment, including its calibration and maintenance of gas chromatographs. Inspection, maintenance, calibration, and repair of all gas chromatographs must be documented in some form to demonstrate a good-quality program. Most manufacturers provide methods to calibrate their instruments. When these methods are modified or other methods are used, complete documentation must be provided. This documentation should include all good-quality assurance parameters to show suitability of the method as well as proper accuracy and precision. All standards that are used in the calibration, or methods, for that matter, must be traceable, preferably to the National Institute of Standards and Technology (NIST). Certificates of traceability must be available and provided on request for discovery. Calibration should cover the entire operating range of the instrument, using three or points of reference. Data should be reported in the International System of Units (SI) (23).

17.6.2 Logbooks

Each gas chromatograph should have a logbook that documents instrument performance and operation, including any maintenance performed on that instrument. Accurate recordkeeping is extremely important for troubleshooting and documentation for proof of proper operation. The instrument log should contain a page that summarizes information about the gas chromatograph, including inventory information such as model number, serial number, and similar information for accessories as data station, and autosampler. The instrument log should contain daily entries about the types and number of samples run, as well as all instrument conditions. It should include notations about routine maintenance, such as septum changes, filter replacements, gas-tank supply changes, and flow adjustments. The daily quality test mixture chromatographic data should also be included in the logbook. Documentation such as, signal-to-noise ratios, detector minimum detectable

quantity, peak retention time, separation and resolution, column plate number, and other related chromatographic data should be recorded as deemed necessary.

The data in this logbook should be archived for easy retrieval and should be periodically reviewed for trends to prevent downtime and for troubleshooting. The data can be used, for example, to determine when to change a column or clean the detector or inlet.

17.6.3 Reports

Suggested wording and report formats should be documented for staff instructions. Key elements for inclusion in the final report as well as statistical data considerations need be included in the documentation. The final report should include documentation of the results obtained, appropriate statistical analyses, and final conclusions. The notes should include documentation of the process to which the sample was subjected, the results obtained, and all data, including the gas chromatographic charts. The report and the notes, including the data, should be subject to a peer and administrative review before being released by the laboratory.

17.6.4 Archiving, Storage, and Retrieval of Documents

It is important that the laboratory have a policy on archiving of data. It is very possible, and in some cases probable, that reports and data may need be retrieved years after which it was generated. Therefore, it is important that all raw data including gas chromatographic charts, documentation, records, protocols, specimens, and final reports that are generated be retained according to laboratory policy. The retention time depends on the type of sample and the regulations pertaining to it, and could range from a typical 2–5 years all the way to 99 years. The responsibility of these archives should be given to a specific person or a group of people designated to be the archivist.

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APPENDIXES

APPENDIX A

Effect of Detector Attenuation Change and Chart Speed on Peak Height, Peak Width, and Peak Area

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I

1. ATTENUATION CHANGE: Inverse proportional change in peak height h and peak area A; that is, increase in attenuation (sensitivity decrease) decreases peak height h and peak area A.

2. CHART SPEED CHANGE: Proportional change in peak width W and peak area A, that is, increase in chart speed increases peak width W, and peak area A.

Example A:

Doubling Chart Speed
Initial conditions: $A_1 = h_1 \times W_1$
Final conditions: $A_2 = h_2 \times W_2$
Note: These conditions will be the same for all examples:
$h_1 = h_2$ but $W_2 = 2W_1$
$A_2 = h^2 \times 2W_1$
Thus $A_2 = 2A_1$

Example B:

Halving Chart Speed
$h_1 = h_2$ but $W_2 = 0.5W_1$
$A_2 = h_2 \times 0.5W_1$
Thus $A_2 = 0.5A_1$

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Example C:

Doubling Attenuation (*Halving Sensitivity*)

$W_1 = W_2$ but $h_2 = 0.5h_1$

$A_2 = 0.5h_1 \times W_2$

Thus $A_2 = 0.5A_1$

Example D:

Halving Attenuation (*Doubling Sensitivity*)

$W_1 = W_2$ but $h_2 = 2h_1$

$A_2 = 2h_1 \times W_2$

Thus $A_2 = 2A_1$

Example E:

Doubling Chart Speed and Attenuation

$W_2 = 2W_1$ and $h_2 = 0.5h_1$

$A_2 = 0.5h_1 \times 2W_1 = h_1 \times W_1$

Thus $A_1 = A_2$

Example F:

Halving Chart Speed and Attenuation

$W_2 = 0.5W_1$ and $h_2 = 2h_1$

$A_2 = 2h_1 \times 0.5W_1 = h_1 \times W_1$

Thus $A_1 = A_2$

Example G:

Double Chart Speed and Halving Attenuation

$W_2 = 2W_1$ and $h_2 = 2h_1$

$A_2 = 2h_1 \times 2W_1 = 4h_1W_1$

Thus $A_1 = 0.25A_2$

Example H:

Halving Chart Speed and Doubling Attenuation

$h_2 = 0.5h_1$ and $W_2 = 0.5W_1$

$A_2 = 0.5h_1 \times 0.5W_1 = 0.25h_1W_1$

Thus $A_1 = 4A_2$

II

1. In general, then, we know that
   a. Each increase in attenuation (decrease in sensitivity) *halves* the previous h or A, that is, 1, 0.5, 0.25, 0.125, 0.0625, and so on.
   b. Each decrease in attenuation (increase in sensitivity) *doubles* the previous h or A, that is, 1, 2, 4, 8, 16, and so forth.

c. Each increase or decrease in chart speed causes a proportional change in W and A.

2. Let $x =$ initial attenuation setting. If we *increase* the attenuation n times, the change in h or A will be

$$x(0.5)^n \times h \quad \text{or} \quad A$$

If we *decrease* the attenuation n times, the change in h or A will be

$$x(2)^2 \times h \quad \text{or} \quad A$$

3. Let $Y =$ initial chart speed and $N =$ final chart speed. If we *increase* the chart speed, the change in W or A will be

$$\frac{N}{Y} \times W \quad \text{or} \quad A$$

If we *decrease* the chart speed, the change in W or A will be

$$\frac{1}{Y/N} \times W \quad \text{or} \quad A$$

APPENDIX B

Gas Chromatographic Acronyms and Symbols and Their Definitions

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ACRONYMS

AAS	Atomic absorption spectroscopy
ACN	Acrylonitrile
ACS	Activated-charcoal strip
AED	Atomic emission spectrometry
AFID	Alkali flame ionization detector
AFS	Ampere(s) full scale
AN	Area normalization
ANRF	Area normalization with response factors
API	Atmosphere pressure ionization
ARF	Absolute response factor
ASE	Accelerated solid extraction
ata	Atmosphere absolute
BAC	Blood alcohol concentration
BET	Brunnauer-Emmett-Teller
BOD	Biochemical oxygen demand
BTU	British thermal unit
BTX	Benzene-toluene-xylene
CCE	Countercurrent extension
CDS	Controlled dangerous substance
CE	Coating efficiency
CEF	Cyanoethylformamide

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cGLP	Current Good Laboratory Practice (regulation)
CI	Chemical ionization
CMS	Carbon molecular sieve
CRF	Chromatographic response function
CV	Coefficient of variation
DCP	Direct-current plasma
DEGS	Di(ethylene glycol) succinate
DHS	Dynamic headspace sampling
DIPE	Di(isopropyl ether)
DMA	Dimethylanaline
DMDCS	Dimethyldichlorosilane
DME	Dimethoxyethane
DPTCP	Di-*n*-propyltetrachlorophthalate
DVB	Divinylbenzene
EA	Electron affinity
EC	Emulsion chromatography
ECD	Electron-capture detector
ECN	Effective carbon number
EDSAC	Endocrine Disruptor Screening Advisory Committee (of USEPA)
EDTA	Ethylenediaminetetraacetic acid
EDXS	Energy-dispersive X-ray spectrometry
EFID	Electrolyzer-powered flame ionization detector
EGDMA	Ethylene glycol dimethacrylate
EI	Electron impact; electron ionization
EIP	Extracted ion profile
ELCD	Electrolytic (Hall) conductivity detector
EMFC	Electronic mass flow controller
EMIT	Enzyme multiplied immunoassay technique
EP	Electropolished
EPC	Electronic pressure control
DPR	Electron paramagnetic resonance
ETBE	Ethyl *tert*-butyl ether
FBP	Final boiling point
FD	Field desorption
FFAP	Free fatty-acid phase
FFF	Field flow fractionation
FIA	Fluorescent indicator adsorption
FID	Flame ionization detector
FPD	Flame photometric detector
FSOT	Fused-silica open tubular column
FTIR	Fourier transform infrared
GABA	γ-Aminobutyric acid
GALP	Good Automated Laboratory practice (regulation)
GBL	γ-Butyrolactone
GCD	Gas chromatographic distillation

GFC	Gel filtration chromatography
GC	Gas chromatography
GHB	γ-Hydroxybutyric acid
GLC	Gas-liquid chromatography
GLP	Good Laboratory Practice (regulation)
GLSC	Gas-liquid-solid chromatography
GPC	Gel permeation chromatography
GRO	Gasoline-range organics
GSC	Gas-solid chromatography
GSGD	Gas-sampling glow discharge
GSR	Gunshot residue
HAFID*	Hydrogen atmosphere flame ionization detector
HCOT	Helically coiled open tubular (column)
HDID	Helium discharge ionization detector
HDPE	High-density polyethylene
HECD	Hall electrolytic conductivity cell
HETP	Height equivalent to a theoretical plate
HIA	Hydride ion affinity
HID	Helium ionization detector
HPLC	High-performance liquid chromatography
HSAS	Headspace autosampler
HSGC	Headspace or high-speed gas chromatography
HSSPME	Headspace sampling and solid-phase microextraction
HT	High temperature
HTS	Hydrogen-transfer system
IBP	Initial boiling point
ICP	Inductively coupled plasma
i.d.	Inner diameter
IDPF	Initial demonstration of proficiency
IE	Ionization energy
IGC	Inverse gas chromatography
IQ	Installation qualification
IRD	Infrared detector
IRS	Infrared spectroscopy
ISP	Internet service provider
IST	Internal standardization technique
LCCO	Light catalytic cycle oil
LCS	Laboratory control sample
LFB	Laboratory fortified blank
LIMS	Laboratory information management system
LLC	Liquid-liquid column (chromatography)
LLE	Liquid-liquid extraction
LMB	Laboratory method blank

*Details on HAFID are presented in *Anal. Chem.* **51**(2), 291 (1979).

LOD	Limit of detection
LOQ	Limit of quantification
LPDE	Low-density polyethylene
LPG	Liquefied petroleum gas
LSC	Liquid-solid chromatography
LUST	Leaking underground storage tank
LVI	Large-volume injection
MAE	Microwave-assisted extraction
MAOT	Maximum allowable operating temperature (for stationary phase)
MAPP	Methylacetylene propadiene
MCLG	Maximum contaminant limit goal
MCR	Metabolic clearance rate
MDA	Methylenedioxamphetamine
MDL	Maximum detectable level (by detector)
MDMA	Methylenedioxymethamphetamine
MEMS	Microelectromechanical system(s)
MESI	Membrane extraction with a sorbent interface
MHE	Multiple-headspace extraction
MIM	Multiple-ion monitoring
MIMS	Membrane introduction mass spectrometry
MIP	Microwave-induced plasma
MLLE	Microscale liquid-liquid extraction
MMLLE	Microporous membrane liquid-liquid extraction
MPD	Microwave plasma detector
MS	Mass spectrometry; matrix spike
MSD	Matrix spike duplicate; mass-selective detector
MSSV	Microscaled seal vessel pyrolysis
MTBE	Methyl *tert*-butyl ether
MW	Molecular weight
NAN	Non-acid-washed
NE	Nelsen-Eggertson
NICI	Negative-ion chemical ionization
NIMS	Negative-ion mass spectrometry
NIST	National Institute of Standards and Technology
NMR	Nuclear magnetic resonance
OCI	On-column injection
o.d.	Outer diameter
O-FID	Oxygen-specific flame ionization detector
OPGV	Optimum practical (carrier-)gas velocity
OQ	Operational qualification
OTC	Open tubular (capillary) column
PA	Proton affinity
PAH	Polycyclic aromatic hydrocarbon
PBM	Probability-based matching
PCB	Poly(chlorinated biphenyl)

PCDD	Poly(chlorinated dibenzo-*p*-dioxin)
PCDF	Poly(chlorinated dibenzo-*p*-furan)
PDECD	Pulse discharge electron-capture detector
PDHID	Pulse discharge helium ionization detector
PDMS	Polydimethylsiloxane
PEG	Poly(ethylene glycol)
PEI	Polyethyleneimine
PEMA	Phenylethylmalonamide
PFE	Pressurized fluid extraction
PFPD	Pulsed-flame photometric detector
PGC	Pyrolysis gas chromatography; process gas chromatograph
$PH(GC)^2$	Pyrolysis hydrogenation with glass capillary gas chromatography
PI	Performance index
PIANO	Paraffin(s)-isoparaffin(s)-aromatic(s)-naphthene(s)-olefin(s)
PICI	Positive-ion chemical ionization
PID	Photoionization detector
PLOT	Porous-layer open tubular column
PME	Polymeric membrane extraction
PMS	Pyrolysis mass spectrometry
PMT	Photomultiplier tube
PONA	Paraffin(s)-olefin(s)-naphthene(s)-aromatic(s)
PPCPs	Pharmaceutical and personal care products
PQ	Performance qualification
PTGC	Programmed-temperature gas chromatography
PTV	Programmed-temperature vaporization
PUF	Polyurethane foam
QA/QC	Quality assurance/quality control
QAP	Quality assurance plan or program
RAN	Raw area normalization
RBCA	Risk-based corrective action
RE	Reaction energy
RF	Response factor
RI	Retention index
RIA	Radioimmunoassay
RMS	Root mean square
RPD	Relative percent difference
RRF	Relative response factor
RRT	Relative retention time
RSD	Relative standard deviation
SAW	Surface acoustic wave
SBR	Styrene-butadiene rubber
SBSE	Stirbar sorptive extraction
SCD	Sulfur chemiluminescence detector
SCF	Standard cubic foot or feet
SCOT	Support-coated open tubular (column)

SEC	Size-exclusion chromatography
SEF	Speed enhancement factor
SEM	Scanning electronmicrograph
SFC	Supercritical-fluid chromatography
SFE	Supercritical-fluid extraction
SHE	Static headspace extraction
SHS	Static headspace sampling
SI	Surface ionization
SIM	Single-or select(ed)(ive)-ion monitoring
SIMDIS	Simulated distillation
SLME	Supported liquid membrane extraction
SMB	Supersonic molecular beam
SN	Separation number
S/N	Signal to noise ratio
SOA	Saturate(s)-olefin(s)-aromatic(s)
SOP	Standard operating procedure
SPCC	System performance check compound
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRC	Solvent refined coal
SS	Surrogate standard; stainless steel
STP	Standard temperature and pressure (25°C and 1 atm)
SVOC	Semivolatile organic compound
SVP	Saturation vapor pressure
TAA	Triamcinolone acetonide
TAME	*tert*-Amyl methyl ether
TCC	Target compound chromatogram
TCD	Thermal conductivity detector
TCEP	1,2,3-Tris(2-cyanoethoxy)propane
TCEPE	Tetracyanoethylated pentaerythritol
TEA	Thermal energy analyzer
TEC	Toxicity equivalent concentration
TEL	Tetraethyl lead
TIC	Total-ion current or chromatogram
TID	Thermionic detector
TIM	Total ion mode
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TOC	Total organic carbon
TOF	Time of flight
TPH	Total petroleum hydrocarbon
TS	Theoretical segment
TSD	Thermionic specific detector (term also used for nitrogen-phosphorus detector)
TSLLE	Thermospray liquid-liquid extraction

2DGC	Two-dimensional gas chromatography
Tz	Trennzahl number
UOP	Universal Oil Products (proprietary name)
UTE	Utilization of theoretical efficiency
VCR	Vacuum-coupled (and) replaceable
VOC	Volatile organic compound
WBOT	Wide-bore open tubular (column)
WCOT	Wall-coated open tubular (column)
WWCOT	Whisker-wall-coated open tubular (column)
WWPLOT	Whisker-wall porous-layer open tubular (column)
XRD	X-ray diffraction

SYMBOLS

General

a	Uptake, in grams per gram of adsorbent; packing porosity
$a^{u}_{s(M)}$	Activity coefficient in stationary phase (mobile)
$A(A_p)$	Peak area; surface area of solid granular adsorbent; van Deemter equation eddy diffusion term
A_c	Cross-sectional area of a column (internal)
B	van Deemter equation molecular diffusion term; second virial coefficient
B_0	Specific permeability
c	Gas-phase concentration
c_a	Adsorbed-phase concentration
C	van Deemter equation mass transfer term
C_G	Concentration of solute component in gas phase
C_i	Concentration of a test substance in mobile phase at detector
C_M	Concentration of solute component in mobile phase
C_{p1}	Gram specific-heat ratio of carrier gas at constant pressure
C_{p2}	Gram specific-heat ratio of sample at constant pressure
C_S	Concentration of solute component in stationary phase
d	Tube diameter
d_c	Column inside diameter
d_f	Thickness of liquid-phase film
d_p	Diameter of support particle
D_A	Density of absorbent
D_c	Concentration distribution ratio
D_g	Distribution coefficient
D_m	Mass distribution ratio
D_s	Distribution coefficient
D_v	Distribution coefficient
D	Minimum detectability of a detector; diffusion coefficient in general, density, distribution ratio

D_G	Diffusion coefficient in gas phase
D_L	Diffusion coefficient in liquid stationary phase
D_M	Diffusion coefficient in mobile phase
D_S	Diffusion coefficient in stationary phase
E^*	Activation energy
f	Relative detector response factor; also frequency
F	Frequency
F_a	Mobile-phase flowrate at ambient temperature
F_c	Mobile-phase flowrate corrected to column temperature
$\overline{\overline{F}}_c$	Average flowrate of mobile phase in column
F_0	Initial flowrate of mobile phase into column
F_s	Split-vent flowrate
$\overline{\Delta G^\circ}$	Free energy of adsorption
h	Reduced plate height; peak height
H	Plate height (height equivalent to one theoretical plate); McReynold's constant for 2-methylpentanol-2
H_{eff}	Effective plate height (height equivalent to one effective plate)
$\overline{\Delta H^\circ_{ST}}$	Isoteric heat of adsorption
I	Retention index
$\Sigma\Delta I$	Sum of McReynolds numbers; used for stationary-phase characterization
I^0	Initial photon flux
IR	Infrared; infrared detector
IST	Internal standard technique
I^T	Retention index obtained in programmed-temperature analysis
j	Compressibility correction factor
J	McReynold's constant for iodobutane
k	Retention factor (capacity factor)
K	Absolute temperature; distribution constant in general; McReynold's constant for 2-octyne
K_c	Distribution constant in which concentration in the stationary phase is expressed as weight of substance per volume of the phase
$\tilde{K}_c$	Equilibrium distribution constant
K_D	Distribution constant
$K^0_{D(R,S)}$	Distribution coefficients on pure-phase R or S
K^0	Thermodynamic distribution constant, Equation 11.5
K_g	Distribution constant in which the concentration in the stationary phase is expressed as weight of substance per weight of the dry solid phase
K_s	Distribution constant in which concentration in stationary phase is expressed as weight of substance per surface area of solid phase
L	Column length; McReynold's constant for 1,4-dioxane
M	Molecular weight
M	McReynold's constant for *cis*-hydrindane

M_i	Mass rate of test substance entering detector
n	Moles of a substance in a mixture; mole fraction
n_{ne}	Required plate number
N	Noise of a detector; Avogadro's number; plate number (number of theoretical plates)
N_{eff}	Effective theoretical plate number
p	Pressure in general; pressure drop; relative pressure $P = p_i/p_o$
p_i	Inlet pressure
p_o	Outlet pressure
p^o	Vapor pressure of a pure substance
p_w	Partial pressure of water at ambient temperature
Δp	Pressure drop across column; $\Delta P = p_i - p_o$
Q	Heat flow
$r_{a/b}$	Relative retention $= k_1/k_2$
r_c	Column tubing radius, i.d.
r_G	Unadjusted relative retention
r_p	Pore radius
R	Gas constant; resistance; retardation factor in column chromatography; fraction of a sample component in mobile phase
R_s	Peak resolution
$1 - R$	Fraction of sample component in stationary phase
s	Rohrschneider constant for pyridine; sound pathlength
s'	McReynold's constant for pyridine
S	Separation factor according to Purnell, Equation 2.82; surface area; detector sensitivity
$\overline{\Delta S^\circ}$	Entropy of adsorption
t	Time in general; analysis time, based on solute component more readily sorbed (see Equation 2.94)
t_M	Mobile phase holdup time; it is also equal to the retention time of an unretained compound; referred to as "air peak"
t_N	Net retention time
t_{ne}	Minimum analysis time
t_0	Injection point time
t_R	Total retention time; absolute retention time
t'_R	Adjusted retention time
t^0_R	Corrected retention time
t^T_R	Total retention time in temperature-programmed analysis
$\overline{t_R}$	Peak elution time
T	Temperature in general
T_a	Ambient temperature
T_c	Column temperature
u	Linear velocity of mobile phase; interstitial velocity of mobile phase; Rohrschneider constant for nitromethane
$\overline{u}$	Mean interstitial velocity of mobile phase; i.e., average linear gas velocity

u_D	Diffusion velocity
u_o	Carrier-gas velocity at column outlet; $= F_c L / V_c$
u'	McReynolds constant for nitropropane
V	Volume in general; interstitial volume of mobile phase
V_A	True adsorbent volume
V_c	Column (tube) volume (cm^3); $V_c = A_c L$
V_{ext}	Extra-column volume
V_g	Specific retention volume at 0°C
V_g^θ	Specific retention volume at column temperature
V_G	Interstitial mobile phase volume (interparticle volume) $= V_l$
V_L	Volume liquid stationary phase
V_M	Holdup volume; i.e., retention volume of nonretained peak; mobile phase holdup volume
V_M^0	Corrected holdup volume for nonretained peak
V_N	Net retention volume
V_0	STP volume of one mole of gas; interparticle volume of column
V_R	Absolute retention volume; peak elution volume
V_R'	Adjusted retention volume
V_R^0	Corrected retention volume
V_S	Volume stationary phase
V_t	Total mobile phase in the column
V_S^T	Specific retention volume in gas–solid chromatography
w_b	Peak width at base
w_h	Peak width at half height
w_I	Peak width at inflection points
W	Mass (weight) in general
W_I	Mass (weight) of a test substance present
W_L	Mass (weight) of liquid phase
W_S	Mass (weight) of stationary phase
x	Rohrschneider constant for benzene
x'	McReynolds constant for benzene
X_s	Mole fraction in stationary phase
y	Rohrschneider constant for ethanol
y'	McReynolds constant for butanol-l
Y	Pen response
z	Rohrschneider constant for methyl ethyl ketone; number of carbon atoms of a n-paraffin eluting before the peak of interest
z'	McReynolds constant for methyl n-propyl ketone
$z + 1$	Number of carbon atoms of a n-paraffin eluting after the peak of interest
Z	Area response factor

Greek

α	Separation factor (relative retardation)
α_G	Unadjusted separation factor (relative retention)
β	Phase ratio
γ	Tortuosity factor, expressing uniformity of support particle size and shape; activity coefficient; specific-heat ratio
ε	Interparticle porosity; $\varepsilon = V_0/V_c$
ε_I	Interstitial fraction
ε_S	Stationary-phase fraction
λ	Packing term, expressing uniformity with which a packed column is filled; thermal conductivity
η	Mobile-phase viscosity; efficiency coefficient of ionization
π	Constant = 3.1416
κ	log k
ν	Reduced mobile-phase velocity
ρ	Hammett constant; density
ρ_L	Density of liquid phase at column temperature
σ	Standard deviation of a Gaussian peak; area occupied by one molecule; Hammett constant; absorption cross section
σ^2	Variance of a Gaussian peak
Φ	Fraction of total solute in a given phase; degrees phase change; flow resistance parameter
$\Phi_{A,S}$	Mole fraction of stationary phases A and S in binary mixture
Φ	Fraction remaining in original phase after extraction
μ	Micron, Dalton (atomic mass unit)

APPENDIX C

Useful Hints for Gas Chromatography

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1. When installing a new instrument, follow the installation instructions from the manufacturer diligently.
2. To prevent changes in retention times, replace your septum regularly; this practice will avoid loss of sample components, leaks, ghost peaks, and ultimately column degradation. Also select a septum within the proper temperature range.
3. Select vial septa materials appropriate for the chemical nature of your sample, your autoinjector, and system performance.
4. If the inlet temperature is not specified in the analytical method, 250°C is a good starting temperature.
5. Use high-quality carrier and detector gases and leak-check your system periodically; always change your gas cylinders when tank pressure reaches 200 psi to avoid delivery of the nonvolatiles such as hydrocarbons into the gas chromatographic flow paths. Always use the appropriate grade of pressure regulators. Always use the appropriate gas-purifying traps for all gases.
6. Always strive to purge the column with oxygen- and moisture-free carrier gas for 15–30 min before heating the gas chromatographic column in order to remove the detrimental presence of air; avoid rapid or ballistic heating.
7. Whenever possible, select a column of the least polarity that provides acceptable separation of the most critical band pair; in general, the more polar columns have a lower upper temperature limit and are more susceptible to water and oxygen-induced degredation.
8. Low-bleed columns promote better chromatographic performance and minimize silicon-containing deposits with a FID and other detectors; the

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more sensitive element-specific detectors will generate elevated bleed levels if the stationary phase contains a heteroatom or functional group (–CN or –F) to which a detector responds in a sensitive fashion and may also require more extensive column-conditioning times.

9. For extended lifetimes of polar columns, always use an oxygen trap in the carrier-gas line.
10. Always cut the ends of a capillary column after insertion through graphite ferrules.
11. If you are having problems with solvent focusing or early-eluting peaks appear broad or distorted in splitless injection, consider using a column with a greater film thickness.
12. Routinely inspect and replace dirty inlet liners; poor peak shape can also be attributed to a dirty liner in addition to column fatigue, active sites, and other factors.
13. Use the correct liner for an injection mode; select the inner diameter of a liner to be commensurate with solvent boiling point, solvent expansion, and injection volume when employing a vaporizing inlet.
14. When peak shapes deteriorate, examine your inlet lines (change often). Redeactivate or replace if necessary; at the same time, cut 0.5 m from the inlet end of the column prior to installation.
15. Always use new ferrules with new columns or injector/detector components.
16. Inspect freshly terminated column ends with a light microscope or other magnifying devices, for a proper cut with no jagged, rough edges; at the same time, inspect ferrules for any damage, cracks, or other irregularities, particularly if a ferrule has been used previously.
17. Retightening of graphite/vespel ferrules by $\frac{1}{4}$ to $\frac{1}{2}$ turn after the first two or three temperature-programmed runs is strongly recommended.
18. Always use graphite/vespel ferrules when connecting a column to a gas chromatography–mass spectrometry interface.
19. Do not pressure or thermally shock a column by disconnecting it while hot or with carrier-gas pressure applied; allow a column to reach ambient temperature and pressure prior to disconnection.
20. Condition a column at either (a) 20°C above the upper temperature specified in a method or (b) the recommended maximum column temperature by the column manufacturer.
21. When storing a capillary column, seal the column ends with a septum, remembering to cut at least 3 cm from each end before reinstallation. For packed columns, cap the ends of the column to prevent air and dust particles from entering the column. Place a column in its shipping box for safe storage.
22. When installing a column into a FID jet, never pass the column through the flame. This will burn both the protective polyimide coating on the capillary column and the stationary phase within the column.

23. Set the FID temperature 20°C above the maximum column temperature employed in the method; 250°C is usually the recommended detector temperature; minimum FID temperature must be 100°C.
24. For maximum FID sensitivity, set the air flowrate 10 times higher than the hydrogen flowrate. The ratio of hydrogen flowrate to the combined carrier-gas and makeup-gas flowrates should be approximately unity.
25. Change ferrules and O-rings during system maintenance.
26. Remember that routine inexpensive maintenance saves time and money in the long run. Keep your instrument logbook up-to-date.
27. When changing several parts of your system, change only one at a time.

OBVIOUS VARIABLES IN YOUR GAS CHROMATOGRAPHIC SYSTEM THAT YOU SHOULD MONITOR ON A REGULAR BASIS

1. Gas pressures.
2. Average linear velocity of the carrier gas.
3. Flowrates of detector gases, split-vent gas flowrates, and the septum purge gas flowrates.
4. The detector range and attenuation.
5. All your temperatures (injector, column, detector, etc.)
6. Check the cleanliness of all gas lines and gas traps. Also check expiration dates of all gas traps.
7. Check your entire chromatographic system routinely for leaks.
8. Always monitor your sample and standard concentrations and also the storage dates and the solvent purities.
9. Keep a moderate inventory of septa, O-rings, liners, and ferrules.
10. Routinely inspect your syringes for leaks and cleanliness. Be sure that the needles are sharp. Autosampler syringes should be changed often.
11. Monitor the appearance of your baselines of all your analyses for problems in your system(s).

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where the statue stood
Of Newton, with his prism and silent face,
The marble index of a mind for ever
Voyaging through strange seas of Thought, alone.

—William Wordsworth (1770–1850)
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